

THE EFFECTS OF EXERCISE ON HEART RATE VARIABILITY IN
HEALTHY SUBJECTS AND CLINICAL POPULATIONS.

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by

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Abstract.

Heart rate variability (HRV) is a sensitive, non-invasive measure of cardiac autonomic modulation and a known risk factor for cardiac event and death in certain populations. The acute effects of exercise are to drastically alter HRV. To a degree, the changes tend to reflect the known rearrangement of autonomic control which occurs during exercise. Data concerning chronic effects of exercise training on HRV are mixed. However, some indication of increases in vagally modulated measures exists. The aim of this thesis was to examine the acute and chronic effects of exercise on short-term measures of HRV in healthy subjects and clinical populations.

Initial investigations assessed the agreement and reliability of different, commercially available measurement systems for short-term HRV. The initial literature review concluded that data were mixed concerning the reliability of short-term measurement. The consequent chapters and papers found that agreement was dependent on the systems used and that reliability was fair under only certain physiological conditions.

Reviewing the literature concerning the chronic effects of exercise on HRV also provided mixed results. However, a meta-analysis of these data confirmed a strong, highly significant main effect for increased global (SDNN) vagal (HF) measures of HRV. This effect was modulated by: subject age, sex and the length of the exercise intervention. Empirical work on healthy adults showed that the application of recommended HRV measures to exercise ECG data was limited mainly to lower intensities. Alternative measurement techniques were also employed with only moderate success.

Prior to this thesis, little was known about the autonomic function of patients with peripheral artery disease (PAD). A randomised controlled trial of exercise training in 52 PAD patients was successfully increased maximal walking time in patients on a supervised walking program but did not alter any resting HRV measures. However, HRV measured during exercise sessions, demonstrated significant increases in global and vagally mediated frequency domain measures.

Changes in HRV in cardiac rehabilitation patients have been examined fairly thoroughly in the past but baseline measures are usually made very soon after MI or surgery. This study into the effects of eight weeks of cardiac rehabilitation on HRV measures showed that measures could be positively affected in patients with a delayed entry to cardiac rehabilitation. Indeed, all expected resting HRV measures increased significantly demonstrating increased vagal modulation of heart rate.

In both PAD and post-MI patients, HF power was found to be a significant predictor of improvement in exercise performance. Previously this relationship has only been shown in healthy subjects.

In conclusion, HRV is a significant risk factor in a number of populations. Exercise is capable of modifying HRV in certain cases. HRV should, therefore, be viewed as a therapeutic target.

TABLE OF CONTENTS.

CHAPTER 1. INTRODUCTION TO THESIS.....	1
1.1. INTRODUCTION.....	1
1.1.1. <i>History.</i>	1
1.1.2. <i>The current position of HRV in clinical and scientific investigation.</i>	1
1.1.3. <i>Current applications of heart rate variability measurements.</i>	1
1.2. AIM.....	2
1.3. STRUCTURE.....	2
1.4. NOTES FORMAT OF THE THESIS.....	4
1.5. REFERENCES.....	4
CHAPTER 2. RELIABILITY OF HEART RATE VARIABILITY MEASUREMENTS: A SYSTEMATIC REVIEW OF THE CURRENT LITERATURE.....	7
2.1. AIM AND METHODOLOGY.....	9
2.2. INTRODUCTION TO HEART RATE VARIABILITY.....	10
2.2.1. <i>Analytical Options.</i>	11
2.2.2. <i>Collection Methods.</i>	14
2.3. REJECTION OF STUDIES.....	14
2.4. THE INTRACLASS CORRELATION COEFFICIENT (ICC).....	19
2.5. STUDIES USING THE INTRACLASS CORRELATION COEFFICIENT.....	20
2.5.1. <i>Ambulatory ECG recordings.</i>	20
2.5.2. <i>Short-term ECG recordings.</i>	21
2.5.2.1. <i>Stable, resting ECG recordings.</i>	21
2.5.2.2. <i>Test/manoeuvre ECG recording.</i>	22
2.5.3. <i>Summary of reliability assessed by intraclass correlation coefficient.</i>	22
2.6. RELIABILITY OF HRV USING COEFFICIENT OF VARIATION (CV), RELIABILITY COEFFICIENT (RC) AND LIMITS OF AGREEMENT (LOG).....	23
2.6.1. <i>Methods.</i>	23
2.6.2. <i>Ambulatory recordings.</i>	24
2.6.3. <i>Resting/Stable ECG monitoring.</i>	28
2.6.4. <i>Test/manoeuvre ECG recording.</i>	32
2.7. DISCUSSION OF FINDINGS.....	35
2.7.1. <i>Introduction.</i>	35
2.7.2. <i>Ambulatory vs. short-term tests – which are more reliable?</i>	36
2.7.3. <i>Time domain vs. frequency domain analysis – which is more reliable?</i>	38
2.7.4. <i>The effect of disease state of study population on reliability.</i>	39
2.7.5. <i>The effect of time from test to retest on reliability.</i>	40
2.7.6. <i>Do certain measures demonstrate lower reliability than others?</i>	41
2.7.7. <i>The appropriate levels of reported accuracy.</i>	42
2.8. CONCLUSIONS AND RECOMMENDATIONS.....	43
2.9. REFERENCES.....	45

CHAPTER 3. AGREEMENT AND RELIABILITY OF THREE DIFFERENT METHODS USED TO ASSESS HEART RATE VARIABILITY.....	55
3.1. INTRODUCTION.....	56
3.1.1. <i>Potential sources of error in HRV analysis.</i>	58
3.1.2. <i>Studies assessing agreement between HRV instrumentation.</i>	59
3.1.3. <i>Studies assessing reliability of HRV instruments.</i>	65
3.2. METHODS.....	67
3.2.1. <i>Study population.</i>	67
3.2.2. <i>Instrumentation and data analysis.</i>	67
3.2.3. <i>Study design.</i>	67
3.2.4. <i>Heart rate variability analysis.</i>	71
3.2.5. <i>Statistical analysis.</i>	72
3.3. RESULTS.....	73
3.3.1. <i>Agreement.</i>	74
3.3.2. <i>Reliability.</i>	83
3.4. DISCUSSION.....	84
3.4.1. <i>Agreement.</i>	84
3.4.2. <i>Reliability.</i>	91
3.4.2.1. <i>Reasons varied CV values.</i>	91
3.4.3. <i>Comparisons with previous data.</i>	92
3.4.4. <i>Recommendations.</i>	95
3.5. CONCLUSIONS.	95
3.5.1. <i>Agreement.</i>	95
3.5.2. <i>Reliability.</i>	96
3.6. REFERENCES.....	97

CHAPTER 4. HEART RATE VARIABILITY AND EXERCISE..... 104

4.1.	INTRODUCTION.....	105
4.2.	ASSESSMENT OF AUTONOMIC FUNCTION IN ATHLETES AND CONTROLS AT REST USING HRV.....	106
4.3.	LONGITUDINAL DATA CONCERNING THE EFFECTS OF EXERCISE ON HRV.....	116
4.3.1.	<i>Studies showing no significant effect of training on HRV.....</i>	<i>117</i>
4.3.2.	<i>Studies showing a significant effect of training on HRV.....</i>	<i>118</i>
4.3.3.	<i>Summary.....</i>	<i>120</i>
4.4.	HEART RATE VARIABILITY DURING EXERCISE.....	120
4.4.1.	<i>The HRV response to exercise – results from studies using general spectral analysis..</i>	<i>121</i>
4.4.1.1.	Spectral measures of HRV during exercise.....	122
4.4.1.2.	Changes in LF% and HF% during exercise.....	123
4.4.1.3.	LFnu, HFnu and LF:HF ratio during exercise.....	124
4.4.1.4.	The role of methodological heterogeneity in the disparity of results.....	126
4.4.1.5.	Reasons for the unexpected behaviour of spectral measures during exercise.....	127
4.4.1.6.	The use of alternative exercise protocols to investigate the behaviour of spectral measures during exercise.....	129
4.4.1.7.	Alternative data treatment.....	130
4.4.1.8.	Summary of findings using GSA during exercise.....	130
4.4.2.	<i>Heart rate variability response to exercise: findings from studies using coarse graining spectral analysis.....</i>	<i>131</i>
4.4.3.	<i>HRV during exercise: Studies using CGSA.....</i>	<i>131</i>
4.4.3.1.	Harmonic oscillations during exercise measured by CGSA.....	132
4.4.3.2.	Summary of findings from CGSA.....	135
4.5.	CROSS-SECTIONAL AND LONGITUDINAL STUDIES EXAMINING DIFFERENCES IN THE HRV RESPONSE TO EXERCISE.....	136
4.5.1.	<i>The usefulness of HRV measures during exercise.....</i>	<i>137</i>
4.6.	SUMMARY.....	138
4.7.	REFERENCES.....	140

CHAPTER 5. DIFFERENCES IN RESTING MEASURES OF HEART RATE VARIABILITY IN FIT AND UNFIT SUBJECTS AS DEFINED BY $\dot{V}O_{2PEAK}$ 151

5.1.	INTRODUCTION.....	152
5.2.	METHODS.....	154
5.2.1.	<i>Subjects.....</i>	<i>154</i>
5.2.2.	<i>Equipment.....</i>	<i>154</i>
5.2.2.1.	Heart rate variability measurement.....	154
5.2.2.2.	Respiratory measurements.....	156
5.2.3.	<i>Protocol.....</i>	<i>157</i>
5.2.3.1.	Assessment of $\dot{V}O_{2peak}$ and CPO_{peak}	157
5.2.4.	<i>Calculations.....</i>	<i>158</i>
5.2.5.	<i>Heart rate variability measurement.....</i>	<i>158</i>
5.2.6.	<i>Measurement of HRV, blood lactate and CPO during incremental exercise.....</i>	<i>159</i>
5.2.7.	<i>Data analysis.....</i>	<i>160</i>
5.2.7.1.	Normality of distribution and homogeneity of variances.....	160
5.2.7.2.	Statistical tests of difference.....	161
5.3.	RESULTS.....	161
5.4.	DISCUSSION.....	164
5.4.1.	<i>Impact of statistical methods.....</i>	<i>167</i>
5.4.2.	<i>Application of findings.....</i>	<i>167</i>
5.5.	REFERENCES.....	170

CHAPTER 6. PATTERNS IN TIME AND FREQUENCY DOMAIN MEASURES OF HEART RATE VARIABILITY IN RESPONSE TO EXERCISE: THE EFFECTS OF BASELINE HRV CHARACTERISTICS IN RESPONSE TO EXERCISE.		174
6.1.	INTRODUCTION.....	176
6.2.	METHODS.....	177
6.2.1.	<i>Subjects</i>	177
6.2.2.	<i>Equipment</i>	178
6.2.2.1.	<i>Heart rate variability measurement</i>	178
6.2.2.2.	<i>Respiratory measurements</i>	180
6.2.3.	<i>Protocol</i>	180
6.2.3.1.	<i>The Assessment of $V_{O_{2peak}}$ and CPO_{peak}</i>	180
6.2.3.2.	<i>Calculations</i>	181
6.2.3.3.	<i>Heart rate variability measurement</i>	182
6.2.4.	<i>Measurement of HRV, blood lactate and CPO during incremental exercise</i>	183
6.2.5.	<i>Data analysis</i>	184
6.2.5.1.	<i>Data treatment and statistical analyses</i>	184
6.2.5.2.	<i>Heart rate variability during exercise</i>	184
6.2.5.3.	<i>The effect of baseline characteristics on heart rate variability during exercise</i>	185
6.3.	RESULTS.....	186
6.3.1.	<i>The effects of exercise on heart rate variability</i>	186
6.3.1.1.	<i>Time domain data</i>	186
6.3.1.2.	<i>Frequency domain data</i>	188
6.3.2.	<i>The effect of subject baseline characteristics of heart rate variability response to exercise</i> <i>190</i>	
6.3.2.1.	<i>Time domain measures</i>	190
6.3.2.2.	<i>Frequency domain measures</i>	194
6.4.	DISCUSSION.....	195
6.4.1.	<i>Heart rate variability during exercise</i>	195
6.4.1.1.	<i>Time domain measures of HRV during exercise</i>	196
6.4.1.2.	<i>Frequency domain measures</i>	197
6.4.2.	<i>Differences in heart rate variability response to exercise in relation to subject baseline characteristics</i>	200
6.5.	CONCLUSIONS.....	202
6.6.	REFERENCES.....	203

**CHAPTER 7. THE DEVELOPMENT OF A MODIFIED, GENERAL SPECTRAL ANALYSIS
HEART RATE VARIABILITY FRAMEWORK FOR USE DURING EXERCISE. 208**

7.1.	INTRODUCTION.....	210
7.1.1.	High frequency power (HF).....	210
7.1.2.	Low frequency power (LF).....	212
7.1.3.	Low frequency to high frequency ratio (LF:HF ratio).....	213
7.1.4.	Alternative methodologies.....	215
7.1.5.	Availability of different heart rate variability analyses.....	217
7.2.	METHODS.....	219
7.2.1.	Subjects.....	219
7.2.2.	Equipment.....	219
7.2.2.1.	Heart rate variability measurement.....	219
7.2.2.2.	Respiratory measurements.....	221
7.2.3.	Protocol.....	222
7.2.3.1.	The Assessment of Vo_{2peak} and CPO_{peak}	222
7.2.4.	Heart rate variability measurement.....	223
7.2.5.	Measurement of HRV, blood lactate and CPO during incremental exercise.....	224
7.2.6.	Data treatment.....	224
7.2.7.	Post-hoc heart rate variability analysis.....	225
7.2.8.	Statistical analysis.....	225
7.2.8.1.	Analysis of complete data set.....	226
7.2.8.2.	Analysis of HI and LO fitness groups.....	226
7.3.	RESULTS.....	226
7.3.1.	Whole group data.....	228
7.3.2.	LO and HI fitness groups.....	231
7.4.	DISCUSSION.....	232
7.4.1.	HF as a vagal index.....	232
7.4.1.1.	HF during exercise – replication of previous data.....	233
7.4.1.2.	Logical validity of HF as a vagal index.....	234
7.4.2.	LF/TP as a sympathetic index.....	235
7.4.2.1.	LF/TP During exercise – replication of previous data.....	235
7.4.2.2.	Logical validity of LF/TP as a sympathetic index.....	236
7.4.2.3.	Problems with LF/TP.....	236
7.5.	CONCLUSIONS AND LIMITATIONS.....	238
7.6.	REFERENCES.....	238

CHAPTER 8. HEART RATE VARIABILITY AND TRAINING INDUCED-BRADYCARDIA: INFERENCE FROM META-ANALYSIS.	247
8.1. INTRODUCTION.....	249
8.2. METHODS.....	251
8.2.1. <i>Search strategy.</i>	251
8.2.2. <i>Criteria.</i>	251
8.2.3. <i>Review process.</i>	252
8.2.4. <i>Analysis of data from cross-sectional study design.</i>	252
8.2.4.1. High frequency power.....	252
8.2.4.2. SDNN.....	253
8.2.4.3. RR interval.....	253
8.2.5. <i>Analysis of data from longitudinal study design.</i>	253
8.2.5.1. High frequency power.....	253
8.2.5.2. SDNN.....	254
8.2.5.3. RR interval.....	254
8.3. STATISTICAL METHODS.....	255
8.3.1. <i>Tests for Heterogeneity.</i>	255
8.3.2. <i>Subgroup comparisons.</i>	259
8.3.2.1. Analysis of moderator variables in cross-sectional data.....	259
8.3.2.2. Analysis of moderator variables in longitudinal data.....	260
8.4. RESULTS.....	261
8.4.1. <i>Heterogeneity of cross-sectional comparisons.</i>	261
8.4.2. <i>Differences in RR-interval.</i>	262
8.4.2.1. Differences in SDNN.....	263
8.4.2.2. Differences in HF.....	263
8.4.3. <i>Longitudinal comparisons.</i>	267
8.4.3.1. Longitudinal changes in RR interval.....	267
8.4.3.2. Effect of moderator variables on changes in RR.....	267
8.4.3.3. Changes in SDNN.....	269
8.4.3.4. The effect of moderator variables on longitudinal changes in SDNN.....	270
8.4.3.5. Longitudinal changes in HF.....	274
8.4.3.6. Effects of moderator variables on longitudinal changes in HF.....	275
8.4.4. <i>Relationships between effect sizes for HRV variables.</i>	275
8.5. DISCUSSION.....	277
8.5.1. <i>Cross sectional analyses.</i>	277
8.5.1.1. Effects of moderator variables on cross-sectional data.....	278
8.5.1.2. Correlations between effect sizes.....	280
8.5.2. <i>Longitudinal analysis.</i>	281
8.5.2.1. Effects of moderator variables on longitudinal data.....	282
8.5.2.2. The effect of moderator variables on RR interval change.....	282
8.5.2.3. Effects of moderator variables on SDNN.....	284
8.5.2.4. Effect of moderator variables on HF.....	285
8.5.2.5. Problems with subgroups analysis.....	286
8.5.3. <i>Correlations between effect sizes.</i>	287
8.5.4. <i>Study limitations.</i>	288
8.6. CONCLUSIONS.....	288
8.6.1. <i>Recommendations.</i>	289
8.7. REFERENCES.....	290

CHAPTER 9. HEART RATE VARIABILITY IN PERIPHERAL ARTERY DISEASE.	296
9.1. INTRODUCTION.....	297
9.1.1. <i>Can exercise training affect resting and exercise measures of HRV?</i>	298
9.1.1.1. The effects of exercise on HRV in healthy and clinical populations.	298
9.1.1.2. Chronic changes in HRV during exercise.....	300
9.1.2. <i>Changes in exercise performance in PAD patients</i>	300
9.1.3. <i>Central vs. peripheral changes: which are responsible for increased walking performance?</i>	301
9.1.4. <i>Other possible mechanisms for improved walking performance.</i>	302
9.2. METHODS.....	304
9.2.1. <i>Study population.</i>	304
9.2.2. <i>Protocol.</i>	304
9.2.2.1. Resting heart rate variability analysis.	304
9.2.2.2. Exercise heart rate variability measurements.	304
9.2.2.3. Treadmill testing protocol	305
9.2.2.4. Test termination criteria.	306
9.2.2.5. Randomisation and group exercise protocols.	306
9.2.3. <i>Statistical analysis.</i>	307
9.2.3.1. Changes in resting measures of heart rate variability.	307
9.2.3.2. Changes in measures of heart rate variability measured during exercise.	308
9.2.3.3. Baseline measures as predictors of maximal walking distance at baseline and change in maximal walking distance at 12 weeks.	308
9.3. RESULTS.....	308
9.3.1. <i>Baseline data</i>	308
9.3.2. <i>Changes in resting HRV due to exercise training</i>	311
9.3.2.1. Changes during exercise at equal absolute exercise intensity.	311
9.3.2.2. Changes during exercise at equal relative exercise intensity.....	313
9.3.3. <i>Predictors of baseline maximal walking time.</i>	313
9.3.4. <i>Predictors of change in maximal walking time in SU group.</i>	313
9.3.5. <i>Predictors of change in maximal walking time in all patients.</i>	314
9.4. DISCUSSION.....	319
9.4.1. <i>Exercise test and HRV data.</i>	319
9.4.2. <i>Changes due to exercise training</i>	320
9.4.2.1. Changes in walking performance and $\dot{V}O_{2\text{ peak}}$ in SU and HB groups.	320
9.4.2.2. Changes in resting HRV in SU and HB groups.....	321
9.4.3. <i>Linear regression analysis</i>	324
9.4.3.1. Predictors of MWT at baseline.....	324
9.4.3.2. Prediction of Δ MWT over 12 weeks of training.	325
9.4.4. <i>Changes in HRV at equal absolute exercise intensity.</i>	327
9.5. CONCLUSIONS.....	329
9.6. REFERENCES.....	330

CHAPTER 10. CHANGES IN RESTING HEART RATE VARIABILITY IN PATIENTS UNDERTAKING EIGHT WEEKS OF CARDIAC REHABILITATION..... 337

10.1.	INTRODUCTION.....	339
10.1.1.	<i>Post-myocardial infarction changes in HRV due to rehabilitation – a brief review of the literature</i>	340
10.1.2.	<i>Chapter aims and justification.</i>	342
10.1.2.1.	Time to entry into cardiac rehabilitation.....	342
10.1.3.	<i>The importance of control groups and appropriate statistical analyses.</i>	344
10.1.3.1.	Baseline resting HRV measures as indicators of adaptation to training	346
10.1.3.2.	The application of findings to clinical populations.....	349
10.1.4.	<i>Hypotheses and structure of consequent sections.</i>	350
10.2.	METHODS.....	350
10.2.1.	<i>Subjects.</i>	351
10.2.1.1.	Control group.....	351
10.2.2.	<i>Protocol.</i>	351
10.2.2.1.	Modified shuttle-walking test procedure.....	352
10.2.2.2.	Measurements of heart rate response.....	353
10.2.2.3.	Measurements of heart rate variability.....	353
10.2.2.4.	Heart rate variability analysis.....	353
10.2.3.	<i>Heart rate variability measurements and statistical analysis for each comparison.</i> ...	354
10.2.3.1.	Changes in autonomic function due to cardiac rehabilitation.....	354
10.2.3.2.	High frequency spectral power as a predictor of change in shuttle walking test performance. 355	
10.2.3.3.	The LF:HF ratio as a predictor of ANS adaptation.....	355
10.3.	RESULTS.....	356
10.3.1.	<i>Patient baseline descriptive characteristics</i>	356
10.3.2.	<i>Change in heart rate variability measures due to cardiac rehabilitation.</i>	360
10.3.3.	<i>High frequency power as a predictor of change in shuttle walking test performance.</i> 360	
10.3.4.	<i>Low to high frequency power ratio as predictor of change in heart rate variability.</i> ...	362
10.3.5.	<i>Paired group analysis.</i>	362
10.3.6.	<i>Pre- and post-test analysis by patient subgroup.</i>	362
10.4.	DISCUSSION.....	363
10.4.1.	<i>Changes in routinely assessed risk factors due to cardiac rehabilitation.</i>	363
10.4.2.	<i>Changes in resting heart rate variability.</i>	364
10.4.2.1.	Changes in spectral measures.....	364
10.4.2.2.	Changes in time domain indices of HRV.....	370
10.5.	CONCLUSIONS.....	375
10.5.1.	<i>Limitations.</i>	377
10.6.	REFERENCES.....	378

CHAPTER 11. SUMMARY..... 386

11.1.	ABSOLUTE MEASURES OF HEART RATE VARIABILITY.....	386
11.2.	NORMALISED MEASURES AND RATIOS IN HEART RATE VARIABILITY.....	388
11.3.	RECOMMENDATIONS.....	391
11.4.	CONCLUSIONS.....	392
11.5.	REFERENCES.....	393

LIST OF FIGURES.

FIGURE 1-1. OUTLINE OF THESIS.....	6
FIGURE 3-1 THE COMPONENTS OF HRV INSTRUMENTATION (ADAPTED FROM KENNEDY 1995).....	58
FIGURE 3-2: CV (%) FOR TF5 FOR ALL HRV MEASURES IN CONDITIONS 1-3.	78
FIGURE 3-3 CV (%) FOR CT FOR ALL HRV MEASURES IN CONDITIONS 1-3.	79
FIGURE 3-4. CV (%) FOR CP FOR ALL HRV MEASURES IN CONDITIONS 1-3.	80
FIGURE 3-6. SUPPOSED (TOP ROUTE) AND ACTUAL (BOTTOM ROUTE) PROCESS OF DATA COLLECTION BY CT INSTRUMENT.	88
FIGURE 3-7. ACTUAL PROCESS OF DATA COLLECTION BY TF5 INSTRUMENT.	88
FIGURE 3-8. ACTUAL PROCESS OF DATA COLLECTION BY CP INSTRUMENT.	88
FIGURE 3-9 IDENTIFICATION OF DIFFERENCES IN DATA COLLECTION AND ANALYSIS.	90
FIGURE 6-1. NN INTERVAL FROM SUPINE REST TO 75% $\dot{V}O_{2\text{PEAK}}$ IN ALL SUBJECTS.	187
FIGURE 6-2. RMSSD FROM SUPINE REST TO 75% $\dot{V}O_{2\text{PEAK}}$ IN ALL SUBJECTS.	187
FIGURE 6-3. SDNN FROM SUPINE REST TO 75% $\dot{V}O_{2\text{PEAK}}$ IN ALL SUBJECTS.	188
FIGURE 6-4. HIGH AND LOW FREQUENCY POWER (RAW UNITS) FROM SUPINE REST TO 75% $\dot{V}O_{2\text{PEAK}}$ IN ALL SUBJECTS.	189
FIGURE 6-5. HIGH AND LOW FREQUENCY POWER (NORMALISED UNITS) FROM SUPINE REST TO 75% $\dot{V}O_{2\text{PEAK}}$ IN ALL SUBJECTS.	189
FIGURE 6-6. NN INTERVAL FROM SUPINE REST TO 75% $\dot{V}O_{2\text{PEAK}}$ IN SUBJECTS CLASSIFIED AS VAGALLY (VA) OR SYMPATHETICALLY (SY) DOMINANT AT REST.	191
FIGURE 6-7. RMSSD FROM SUPINE REST TO 75% $\dot{V}O_{2\text{PEAK}}$ IN SUBJECTS CLASSIFIED AS VAGALLY (VA) OR SYMPATHETICALLY (SY) DOMINANT AT REST.....	191
FIGURE 6-8. SDNN FROM SUPINE REST TO 75% $\dot{V}O_{2\text{PEAK}}$ IN SUBJECTS CLASSIFIED AS VAGALLY (VA) OR SYMPATHETICALLY (SY) DOMINANT AT REST.....	192
FIGURE 6-9. HF POWER FROM SUPINE REST TO 75% $\dot{V}O_{2\text{PEAK}}$ IN SUBJECTS CLASSIFIED AS VAGALLY (VA) OR SYMPATHETICALLY (SY) DOMINANT AT REST.....	192
FIGURE 6-10. LF POWER FROM SUPINE REST TO 75% $\dot{V}O_{2\text{PEAK}}$ IN SUBJECTS CLASSIFIED AS VAGALLY (VA) OR SYMPATHETICALLY (SY) DOMINANT AT REST.	193
FIGURE 6-11. HFNU FROM SUPINE REST TO 75% $\dot{V}O_{2\text{PEAK}}$ IN SUBJECTS CLASSIFIED AS VAGALLY (VA) OR SYMPATHETICALLY (SY) DOMINANT.	193
FIGURE 6-12 LFNU FROM SUPINE REST TO 75% $\dot{V}O_{2\text{PEAK}}$ IN SUBJECTS CLASSIFIED AS VAGALLY (VA) OR SYMPATHETICALLY (SY) DOMINANT.	195
FIGURE 7-1 CHANGES (MEAN \pm SD) IN RR INTERVAL DURING INCREMENTAL EXERCISE.....	227
FIGURE 7-2 CHANGES (MEAN \pm SD) IN HF POWER (0.1-0.5Hz) DURING INCREMENTAL EXERCISE.	228
FIGURE 7-3 CHANGES (MEAN \pm SD) IN LF/TP DURING INCREMENTAL EXERCISE.	230
FIGURE 7-4 CHANGES (MEAN \pm SD) IN HF DURING INCREMENTAL EXERCISE IN HI AND LO FITNESS GROUPS.	230
FIGURE 7-5 CHANGES (MEAN \pm SD) IN LF/TP DURING INCREMENTAL EXERCISE IN HI AND LO FITNESS GROUPS.	231
FIGURE 8-1 PROCESS FOR THE DETERMINATION OF EFFECT SIZE HETEROGENEITY BY SUBGROUP ANALYSIS.	258
FIGURE 8-2 CROSS-SECTIONAL COMPARISON OF RR INTERVAL BETWEEN TRAINED SUBJECTS/ATHLETES AND INACTIVE CONTROLS.	264
FIGURE 8-3 CROSS-SECTIONAL COMPARISON OF SDNN BETWEEN TRAINED SUBJECTS/ATHLETES AND INACTIVE CONTROLS.	265

FIGURE 8-4. CROSS-SECTIONAL COMPARISON OF HF POWER BETWEEN TRAINED SUBJECTS/ATHLETES AND INACTIVE CONTROLS.	266
FIGURE 8-5. LONGITUDINAL ANALYSIS OF CHANGE IN RR INTERVAL DUE TO EXERCISE TRAINING.	271
FIGURE 8-6. LONGITUDINAL ANALYSIS OF CHANGE IN SDNN DUE TO EXERCISE TRAINING.	272
FIGURE 8-7. LONGITUDINAL ANALYSIS OF CHANGE IN HF POWER DUE TO EXERCISE TRAINING.	273
FIGURE 9-1. MEAN RR INTERVAL DURING EXERCISE AT THE SAME ABSOLUTE EXERCISE INTENSITY AT WEEKS ONE, SIX AND TWELVE.	315
FIGURE 9-2. TOTAL SPECTRAL POWER DURING EXERCISE AT THE SAME ABSOLUTE EXERCISE INTENSITY AT WEEKS ONE, SIX AND TWELVE.	316
FIGURE 9-3 HIGH FREQUENCY SPECTRAL POWER (LOG UNITS) DURING EXERCISE AT THE SAME ABSOLUTE EXERCISE INTENSITY AT WEEKS ONE, SIX AND TWELVE.....	317
FIGURE 9-4 RATIO OF LOW FREQUENCY TO TOTAL SPECTRAL POWER DURING EXERCISE AT THE SAME ABSOLUTE EXERCISE INTENSITY AT WEEKS ONE, SIX AND TWELVE.	318

LIST OF TABLES.

TABLE 2-1 TIME AND FREQUENCY DOMAIN MEASURES OF HRV.....	13
TABLE 2-2 STUDIES REJECTED FROM REVIEW DUE TO INAPPROPRIATE STATISTICAL ANALYSIS.....	15
TABLE 2-3 STUDIES INTO THE RELIABILITY OF HRV MEASURES USING AMBULATORY ECG DATA COLLECTION.....	25
TABLE 2-4 STUDIES INTO THE RELIABILITY OF HRV MEASURES USING SHORT-TERM ECG COLLECTION METHODOLOGY.....	29
TABLE 3-1 TECHNICAL SPECIFICATIONS OF THE THREE HRV INSTRUMENTS USED.....	69
TABLE 3-2. MEAN (\pm SD) SCORES AND RELIABILITY OF ALL INSTRUMENTS IN DATA COLLECTION CONDITION 1: LYING SUPINE WITH FREE BREATHING.....	75
TABLE 3-3. MEAN (\pm SD) SCORES AND RELIABILITY OF ALL INSTRUMENTS IN DATA COLLECTION CONDITION 2: STANDING WITH FREE BREATHING.....	76
TABLE 3-4: MEAN (\pm SD) SCORES AND RELIABILITY OF ALL INSTRUMENTS IN DATA COLLECTION CONDITION 3: LYING SUPINE, CONTROLLED BREATHING (12 BREATHS.MIN ⁻¹).....	77
TABLE 3-5. INTRAClass CORRELATIONS COEFFICIENTS FOR RELIABILITY OF EACH INSTRUMENT IN EACH CONDITION.....	82
TABLE 5-1. DESCRIPTIVE CHARACTERISTICS OF SUBJECTS DIVIDED BY FITNESS LEVEL.....	162
TABLE 5-2. PARAMETRIC ANALYSIS OF HEART RATE VARIABILITY BETWEEN HIGH AND LOW FITNESS LEVEL SUBJECTS.....	162
TABLE 5-3. NON-PARAMETRIC ANALYSIS OF SELECTED HRV MEASURES.....	163
TABLE 6-1. DESCRIPTIVE STATISTICS FOR ALL SUBJECTS (MEAN \pm SD).....	186
TABLE 7-1. POPULATION CHARACTERISTICS OF THE LO AND HI FITNESS GROUPS.....	229
TABLE 8-1 THE EFFECT OF MODERATOR VARIABLES ON EFFECTS SIZES FOR CHANGES IN RR INTERVAL...	268
TABLE 8-2 THE EFFECT OF MODERATOR VARIABLES ON EFFECTS SIZES FOR CHANGES IN SDNN.....	269
TABLE 8-3. THE EFFECT OF MODERATOR VARIABLES ON EFFECTS SIZES FOR CHANGES IN HF POWER.....	274
TABLE 8-4. RELATIONSHIPS BETWEEN VARIABLES FOR ALL ANALYSES.....	275
TABLE 8-5. EFFECTS OF DIFFERENT FITS ON PERCENTAGE VARIANCE EXPLAINED BETWEEN MEASURES. ...	276
TABLE 9-1. DESCRIPTIVE CHARACTERISTICS BY GROUP.....	309
TABLE 9-2 EXERCISE TEST DATA AT BASELINE, SIX AND TWELVE WEEKS.....	310
TABLE 9-3. HEART RATE VARIABILITY MEASURES AT BASELINE, SIX AND TWELVE WEEKS.....	312
TABLE 10-1 REPORTED TIME FROM MYOCARDIAL INFARCTION OR SURGERY TO BASELINE ASSESSMENT FOR ENTRY INTO CARDIAC REHABILITATION.....	343
TABLE 10-2 DESCRIPTIVE CHARACTERISTICS OF CARDIAC REHABILITATION AND CONTROL GROUP SUBJECTS AT BASELINE.....	357
TABLE 10-3 PHYSIOLOGICAL AND PSYCHOLOGICAL EFFECTS OF CARDIAC REHABILITATION.....	358
TABLE 10-4 CHANGE IN HRV MEASURES OF HEART RATE VARIABILITY FOR CARDIAC REHABILITATION AND CONTROL GROUPS.....	359
TABLE 10-5 SUMMARY OF STEPWISE MULTIPLE REGRESSION ANALYSIS FOR LN Δ SWT%.....	361
TABLE 10-6 SUMMARY OF STEPWISE MULTIPLE REGRESSION ANALYSIS FOR LN Δ SWTM.....	361

LIST OF APPENDICES.

APPENDIX I. List of standard heart rate variability abbreviations, definitions and explanations of variables.....395

APPENDIX II. Expanded descriptions and definitions of HRV terminology – including those used for HRV measurement during exercise.....397

APPENDIX III. Example sample size calculations based on reliability coefficients in chapter two.....400

III.I. Sample size calculations.400

APPENDIX IV. Meta-Analysis of effect sizes for changes in global HRV and measures of sympathovagal balance.395

IV.I. Study selection.395

IV.II. Statistical analysis.395

IV.III. Meta-analysis results.....396

IV.IV. Discussion.397

APPENDIX V. Subgroup analysis for chapter ten.400

Figures VIII – XIV - Differences in Δ HRV values for MI and non-MI subgroups.406

V.I. Differences in Δ HRV between subgroups.406

APPENDIX VI. Published papers, selected abstracts and posters.408

LIST OF APPENDICES.....396

List of Figures and Tables in Appendices.

Figure IV-I Changes in SDNN due to cardiac rehabilitation.....403

Figure IV-II Changes in LF:HF due to CR.....404

Figures VI-1 – VI-7
Differences in baseline HRV measures in MI and non-MI Patients.....407-410

Figures VI-8 – VI-14
Differences in HRV response to CR in MI and non-MI patients.....411-413

Table III-1 Sample size calculations for LF / SDNN in post-MI patients...400

Table III-2 Sample sizes for SDNN in post-MI patients.....401

Table III-3 Sample sizes for LF in heart failure patients.....401

Table IV-1 Sample Sizes based on previous data.....406

LIST OR ABBREVIATIONS AND GLOSSARY OF TERMS.

All abbreviations for specific heart rate variability measures are located in appendix one with brief explanations. A full glossary of these terms with expanded explanations is located in appendix two.

List of abbreviations.

ABI	Ankle to brachial blood pressure index
ACE	Angiotensin converting enzyme
ACM	All cause mortality
AHA	American heart association
ARB	Angiotensin receptor blocker
ACSM	The American College of Sports Medicine
BMI	Body mass index.
BP	Blood pressure
CABG	Coronary artery bypass grafting
CAD	Coronary artery disease
CE	Cardiac event
CPO	Cardiac power output
CV	Coefficient of variation
CHF	Chronic heart failure
DBP	Diastolic blood pressure
DCM	Dilated cardiomyopathy
ECG	Electrocardiogram
EDD	End diastolic diameter

EDV	End diastolic volume
ESD	End systolic diameter
EF(%)	Ejection fraction
ESV	End systolic volume
HAD	Hospital anxiety and depression inventory
HRV	Heart rate variability
HUT	Head up tilt
IC	Intermittent claudication
ICC	Intraclass correlation coefficient
IDCM	Idiopathic dilated cardiomyopathy
LVAD	Left ventricular assisting device
LVD	Left ventricular dysfunction
LVESV	Left ventricular end systolic volume
MAP	Mean arterial pressure
MET	Metabolic equivalent
MI	Myocardial infarction
MSNA	Muscle sympathetic nerve activity
MWC	Maximal work capacity
MWT	Maximal walking time
NA	Nor-adrenaline
NYHA	New York Heart Association
PAR	Pulmonary arteriolar resistance

PAD	Peripheral artery (or arterial) disease
PTCA	Percutaneous transluminal coronary angioplasty.
PVCs	Premature ventricular contractions
RPE	Ratings of perceived exertion
RSA	Respiratory sinus arrhythmia
SCD	Sudden cardiac death
SWT	Shuttle walking test
VPC	Ventricular premature contractions

Glossary of terms.

Ambulatory monitoring	Continual recording of the ECG or blood pressure using a recording device worn by the subject during normal daily activities for 24 hours
Ankle to brachial blood pressure index	The ratio of systolic and diastolic blood pressures measured at the ankle and the upper arm – an indicator of severity of PAD.
Baroreflex sensitivity.	The reactivity of the arterial baroreflex to alter blood pressure - usually in response to orthostatic challenge
Body mass index	The ratio of weight (kg) to body size (calculate as stature in m ²)
Borg Scale	6 – 19 point scale providing subject self reported ratings of perceived exertion.
Bridging to recovery	The use of an LVAD to allow the dilated myocardium of a CHF patient to recover.
Bridging to transplantation	The use of an LVAD to keep a patient alive until a suitable donor heart becomes available for transplantation.
Cardiac power output	The flow of blood from the heart in a given time period ($l \cdot \text{min}^{-1}$)
Cardiothoracic Ratio	The transverse cardiac diameter (the horizontal distance between the most rightward and leftward borders of the heart seen on a postero-anterior (PA) chest radiograph) divided by the transverse chest diameter.
Claudication	Ischaemic burning or cramping pain usually in the lower limb of patients with restricted blood flow as in PAD. See also intermittent claudication
Coronary artery bypass grafting	Operation to reroute blood flow from blood vessels of the heart using veins removed from other parts of the body
End diastolic diameter	Geometrical measure of the heart showing the

	diameter of the left ventricle at the end of diastole (mm)
End diastolic volume	The volume of blood in the ventricle at the end of diastole (ml)
End systolic diameter	Geometrical measure of the heart showing the diameter of the left ventricle at the end of diastole (mm)
Ejection fraction	The fraction or % of blood (usually in the left ventricle) at the end of systole as a function of the volume during diastole.
Holter monitor	Recording device worn by subject to continually monitor ECG and/or blood pressure.
Iodine-123 metaiodobenzylguanidine imaging	The infusion and monitoring of iodine-123 metaiodobenzylguanidine to observe the distribution of sympathetic nervous tissue.
Left ventricular end systolic volume	Volume of blood remaining in the left ventricle at the end of systole.
Percutaneous transluminal coronary angioplasty.	Operation to increase blood flow in (coronary) blood vessels by increasing the internal diameter of the vessel. May involve stenting.
Peripheral bypass surgery	Rerouting blood flow around damaged or occluded vessels using grafts from other healthy vessels.
Premature ventricular contractions	Spontaneous depolarization of the ventricular myocytes prior to and without stimulation from the SA node resulting in ventricular contraction too early in the normal cardiac cycle – sometimes VPC.
Stenting	Insertion of a device into a previously occluded blood vessel to hold back plaque built up due to CAD.

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I dedicate this thesis to the memory of my dad and hope the findings presented here and those which we make in the future, will help in the prevention and rehabilitation of the many forms of coronary heart disease.

Gavin Sandercock.

Author declaration.

I take responsibility for all the material contained within this thesis and confirm that it is my own work.

Gavin Sandercock

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CHAPTER 1. INTRODUCTION TO THESIS.

1.1. Introduction.

1.1.1. *History.*

Over twenty years ago, the now seminal paper by Akselrod and colleagues (Akselrod *et al.* 1981) was published in the journal *Science*. In this paper it was stated that. ‘*Sympathetic and parasympathetic nervous activity make frequency-specific contributions to the heart rate power spectrum.*’ The ability of short-term measurements to assess cardiac autonomic modulation from an ECG recording accurately and non-invasively was realised and a new research tool; short-term heart rate variability (HRV) was placed in the public domain.

1.1.2. *The current position of HRV in clinical and scientific investigation.*

A search of the PubMed database in April 2005 for ‘heart rate variability’ produced 7933 hits for these MeSH terms. This is evidence for the appeal a non-invasive and cost effective measure of cardiac autonomic modulation has for clinicians and researchers. The applications of HRV measurements range from its beginnings in foetal heart rate monitoring, through psychological measures, to physiological stressors such as exercise, altitude and space exploration. However, where the application of HRV to research has been most abundant and arguably most successful, is in diagnosis and monitoring of diseases affecting the autonomic nervous system (ANS).

1.1.3. *Current applications of heart rate variability measurements.*

The 1996 Taskforce report on non-invasive electrocardiology (Taskforce 1996) made an evidence-based recommendation to the effect that HRV could be routinely used for assessment of cardiac autonomic function in patients with diabetic autonomic neuropathy and in post-myocardial infarction patients. The report also provided a series of recommendations relating to the technical and

experimental requirements for the recording and analysis of ECG signals for determining HRV. Despite this global standard, many published reports fail either to provide pertinent information regarding data collection or do not meet the required standards set out by the Taskforce.

In this project, the standards of all recording devices and analysis software were in excess of those suggested by the Taskforce. Additionally, all HRV measurements were made within the confines of the conditions set out in the report. The only exceptions to this were the exploratory measurements made under conditions of steady-state exercise. This is not a previously recommended use of HRV despite the fact that it has received much attention in the literature.

Acute changes in HRV reflect shifts in autonomic modulation of the SA node. They describe changes in vagal and sympathetic nervous system function well under resting conditions, particularly when assessed in the same subjects under differing conditions (Pagani *et al.* 1986). The acute effects of exercise are less clear. At low intensities, it appears that changes in HRV reflect vagal withdrawal and sympathetic activation. At higher intensities, where there is little variation in heart period, data are more mixed (Casadei *et al.* 1995). Longitudinal studies suggest that changes in some measures of HRV may be brought about by chronic exercise training (Davy *et al.* 1997), although again the data are mixed (Carter *et al.* 2003). Little information is available concerning the effects of chronic exercise training on the acute response (Myslivecek *et al.* 2002).

1.2. Aim.

The aim of this thesis was, therefore, to observe acute and chronic changes in short-term measures of HRV in healthy subjects and clinical populations.

1.3. Structure.

The Taskforce report gives information on many aspects of HRV but fails to provide a sound review of the literature concerning the reliability of HRV

measurements. Recognising the importance of reliability when a measurement is made longitudinally, chapter two of this thesis was a review of the substantial body of literature on this topic. Chapter three assesses the reliability of the instruments to be used for data collection within this thesis and the agreement between them. Chapter four provides a systematic review of the literature surrounding both the acute and chronic responses of HRV measurements to exercise and exercise training respectively. In order that definite conclusions can be drawn from these data, chapter eight builds on this review by providing a full meta-analysis of both cross-sectional differences in selected HRV measures between groups based on activity levels and assessed longitudinal adaptations to exercise training.

Chapters five to seven provide laboratory based, empirical data to demonstrate:

- i. Cross sectional differences in HRV measures in healthy subjects of differing fitness levels.
- ii. The acute effects of exercise on HRV measures.
- iii. The ability of modified HRV measures to represent acute autonomic adaptation to exercise more accurately.

The application of the knowledge gained in the first eight chapters to clinical populations begins in chapter nine. Where both the standard resting measurements discussed previously, and the modified HRV measures developed for use during exercise in chapter six, are applied to data collected on peripheral artery disease patients. This chapter (nine), assesses the impact of a randomised controlled trial of supervised and home based walking exercise on functional outcomes and HRV measures in these patients with potentially low initial levels of HRV. No previous data concerning the HRV profiles of PAD patients was available at the time of this study.

Chapter 10 also observes change in HRV measures due to exercise intervention but this time in post-myocardial infarction and coronary artery disease patients. This chapter uses a quasi-experimental design in which volunteers attending a cardiovascular rehabilitation programme are studied longitudinally. Data concerning changes in HRV programmes were available prior to the start of this study. However, these were heterogeneous in nature and often methodologically

flawed. Additionally, many studies had utilised 24-hour measurements, taken in patients very soon after MI. This study is therefore unique in using short-term measurements, made in patients made after the well documented (Bigger *et al.* 1992) acute phase of HRV derangement following myocardial infarction.

Chapter 11 summarises the findings of this thesis.

1.4. Notes format of the thesis.

This thesis is written in the modern style. Each chapter represents a discreet piece of work which is linked to the other chapters by a common theme (Figure 1.1). In some cases (chapters four, five and six) a common methodology and population were used. To avoid the reader having to read repeated methodology these sections are *italicised*. References have been placed at the end of each chapter.

1.5. References.

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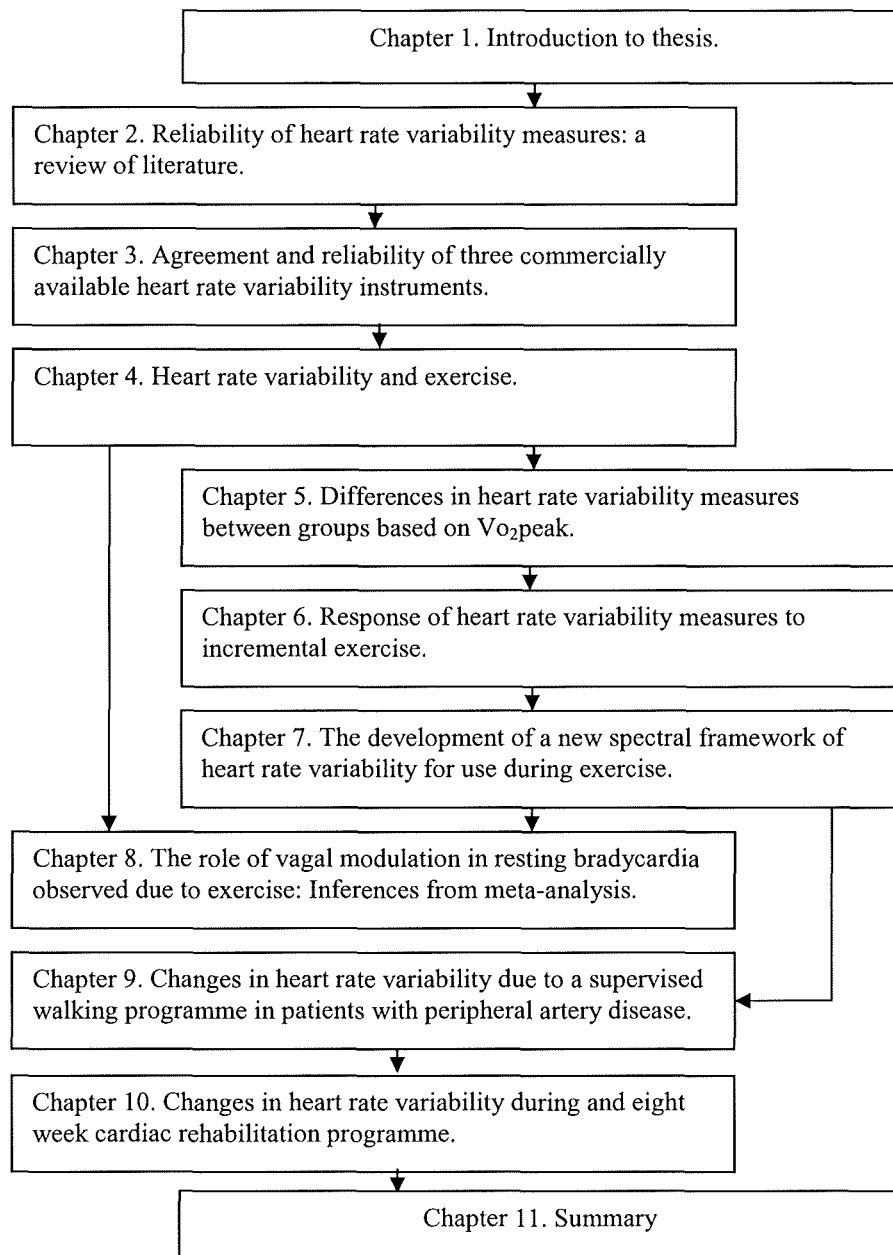


Figure 1-1. Outline of thesis.

CHAPTER 2. RELIABILITY OF HEART RATE VARIABILITY MEASUREMENTS: A SYSTEMATIC REVIEW OF THE CURRENT LITERATURE.

Abstract

Heart rate variability (HRV) has been used as a simple, non-invasive technique to examine autonomic nervous function. Depressed levels of HRV have been shown to be present in a number of pathological conditions including diabetes and following myocardial infarction. Reduced HRV has been shown to be a good predictor of cardiac events including death in a number of these conditions as well as in the elderly and the general population. Within the literature, HRV is commonly referred to as a reliable measurement technique. The aim of this review was to assess the accuracy of this description based upon a comprehensive review of the available data concerning reliability of HRV.

From a search of the literature, it was first determined that numerous, published studies in this area have utilised statistical analyses other than those recommended for the assessment of reliability. By reviewing studies using appropriate statistical analyses it was determined that reliability coefficients for HRV measures were highly variable. Coefficients of variation ranged from <1% to >100%. Similar variation was found in studies using the intraclass correlation coefficient values, and limits of agreement.

In an attempt to determine reasons for some of the heterogeneity of reliability coefficients, several pertinent questions were set. Attempts were then made to provide answers to these statements supported by the literature. Briefly, the literature lends support to the following statements:

- i. Ambulatory ECG recordings monitoring may be a more reliable HRV measurements when compared with those derived from short-term, steady state ECG trace.
- ii. There seems to be little difference between the reliability coefficients reported for either time or frequency domain HRV measures.

- iii. Clinical populations seem consistently to generate less reliable HRV measurements than healthy counterparts.
- iv. Data concerning the effect from test-retest duration on reliability are inconsistent. Although some data suggest longer durations may decrease reliability other studies show no such effect.
- v. One time domain measure that shows some evidence of being less reliable than others is pNN50%. This is especially true in clinical populations, and may be due to the often-low values of this specific measure. In the frequency domain, reliability of LF and HF power are commonly reported concurrently. In a number of studies LF demonstrates inferior reliability.

It can be concluded that describing HRV as a reliable measurement technique is a gross oversimplification as results of reliability studies are highly heterogeneous, and dependent on a number of factors. It is recommended that authors report in-house coefficients for HRV measurements used unless methodologically similar coefficients have been published previously. Lastly, the effects of measurement reliability should be taken into account when deriving sample sizes in longitudinal study designs.

This chapter, in truncated form has been e-published in the International Journal of Cardiology, see appendix VI.

2.1. Aim and Methodology.

For interindividual differences to be assessed accurately and for intraindividual change to be monitored, the method used must also be reliable. Reliability has a number of definitions and for a full review of the concept the reader is directed to an excellent review article (Atkinson and Nevill 1998). In the case of the application of a measurement such as heart rate variability (HRV) the researcher or clinician should be interested in how much a measurement taken on an individual or group varies when it is repeated. This is akin to absolute reliability (Baumgartner 1989), defined as the degree to which repeated measurements vary for individuals.

This review will focus on studies that have assessed the reliability of HRV measures. The electronic databases on Medline and Ovid were searched for studies using the following terms: heart rate variability, reliability, reproducibility. From the references generated by this search strategy a manual search was made for further relevant literature. Inclusion criteria were as follows: the study was undertaken with the specific aim of assessing the reliability of HRV. Studies were required to have used repeated measurements to examine reliability. The investigators were required to have given a methodological account of standardised procedures used to control for possible confounding variables which may have affected reliability.

Critical analysis of statistical methods used to assess reliability was undertaken and only studies using appropriate statistical analysis, as defined by Atkinson *et al.* (1998) were included. Twenty-five potential studies were identified. Of these, seven studies were rejected for methodological reasons.

Table 2-2 contains those studies which have examined reliability but are not included in the main discussion of the topic due to rejection on methodological grounds. Some of these studies still provide insight into HRV reliability, therefore some discussion of their findings as well as reasons for rejection can be found below. Tables 2-3 and 2-4 give brief descriptions of studies which have examined the reliability of HRV measures obtained from ambulatory and ECG

recordings respectively. An explanation of the different methodologies used in HRV sampling is given below.

2.2. Introduction to heart rate variability.

It is commonly perceived that a regular heartbeat is a sign of cardiac health. In truth however, the rhythm of a healthy heart is characterised by significant beat-to-beat variability (Routledge *et al.* 2002). This heart rate variability (HRV) has been used as a simple, non-invasive technique to examine autonomic nervous function (Pagani *et al.* 1986). Depressed levels of HRV have been shown to be present in a number of pathological conditions including: heart disease (Bigger *et al.* 1992; Weber *et al.* 1999), heart failure (Casolo *et al.* 1989; Scalvini *et al.* 1998), diabetes (Malpas and Maling 1990; Ewing *et al.* 1991; Burger *et al.* 1997), hypertension (Konrady *et al.* 2001), asymptomatic left ventricular dysfunction (Scalvini *et al.* 1998), and following myocardial infarction (Kleiger *et al.* 1987; Bigger *et al.* 1991; Bigger *et al.* 1992; Bigger *et al.* 1992).

More importantly reduced HRV has been shown to be a good predictor of cardiac events including:

- a) death in coronary artery disease (van Boven *et al.* 1998)
- b) heart failure (Nolan *et al.* 1998)
- c) stable angina pectoris (Lanza *et al.* 1997; Forslund *et al.* 2002) ,
- d) following myocardial infraction (Kleiger *et al.* 1987; Bigger *et al.* 1993; Bigger *et al.* 1993; Reinhardt *et al.* 1996; Zuanetti *et al.* 1996; La Rovere *et al.* 1998; Huikuri *et al.* 2000; Sosnowski *et al.* 2002; Filipovic *et al.* 2003).
- e) the healthy, elderly population (Tsuji *et al.* 1994).
- f) the general population (Tsuji *et al.* 1996).

As well as these clinical applications, differences in HRV measures have been demonstrated cross-sectionally in numerous epidemiological studies. Measures of HRV are known to differ significantly according to age (Byrne *et al.* 1996; Stein *et al.* 1997; Sinnreich *et al.* 1998; Tulppo *et al.* 1998; Barnett *et al.* 1999; Kuo *et al.* 1999; Fukusaki *et al.* 2000; Kuch *et al.* 2001), sex (Stein *et al.* 1997; Sinnreich *et al.* 1998; Barnett *et al.* 1999; Kuch *et al.* 2001) and habitual levels of

physical activity (Gregoire *et al.* 1996; Tulppo *et al.* 1998; Migliaro *et al.* 2001). Recently there are also limited longitudinal data to suggest HRV can be modified by increased physical activity (Pardo *et al.* 2000; Melanson and Freedson 2001; Portier *et al.* 2001) although findings are inconsistent (Loimaala *et al.* 2000; Hedelin *et al.* 2001).

When the major predictors of age and sex are controlled for, numerous other factors have been shown to account for some of the variance in HRV. Such factors include: body mass index (BMI), height, high-density lipoprotein (HDL) levels, plasma insulin levels, smoking, alcohol and caffeine consumption (Byrne *et al.* 1996; Singh *et al.* 1999; Kuch *et al.* 2001).

2.2.1. *Analytical Options.*

In the above text, HRV is referred to as a single entity. In fact what is being referred to is the cyclical change in sinus rate (the rate of firing of the sinoatrial node) over time (Ori *et al.* 1992). This is a simplistic description a highly complex phenomenon. There are two major approaches to the analysis of HRV. These are time domain analysis and frequency domain analysis. Time domain analysis is the statistical analysis of fluctuations in heart rate based on differences between RR intervals over time, usually 24 hours. Frequency domain analysis is mathematically more complex and involves the decomposition of the RR interval variation data into its frequency components. These are then quantified in terms of their relative intensity, termed power. This type of analysis is also known as power spectral density analysis because the relative energy content of the time series signals of the frequency bands is quantified giving a measure of energy distribution over frequency (Ori *et al.* 1992).

In the simplest terms, alterations in heart rate are controlled by the sympathetic and parasympathetic branches of the autonomic nervous system (ANS). These two branches apply their effect at different frequencies. The parasympathetic system appears to influence the high frequency spectrum (HF, 0.40 – 0.15 Hz) and the sympathetic system appears to influence the low frequency spectrum, (LF, 0.04 – 0.15 Hz) although some parasympathetic activity is also detected

here. Recently there has been much discussion concerning what is represented by these two frequency bands (Lanza *et al.* 1998; Melanson and Freedson 2001), particularly the LF band. Empirical evidence suggests that factors other than sympathetic nervous activity have significant impact on power in the LF spectrum (Chess *et al.* 1975; Goldsmith *et al.* 1992; Lombardi *et al.* 1996; van de Borne *et al.* 1999).

There is also fluctuation in heart rate at much lower frequencies such as very low frequency (VLF, 0.0033-0.04) and ultra low frequency (ULF<0.0033 Hz). The physiological explanation of these components is much less well defined. It is not the purpose of this paper to give a full account of the various spectra or the physiological correlates of each spectra as several excellent reviews on this topic already exist (Taskforce 1996; Stein and Kleiger 1999; Routledge *et al.* 2002).

Time domain analysis creates a number of measures. Table 2-1 gives brief descriptions of time and frequency domain measures recommended for use by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (Taskforce 1996).

Table 2-1 Time and Frequency domain measures of HRV.

Abbreviation	Description	What the measure represents.
Time domain measures.		
SDNN	The standard deviation of all the normal-to-normal (NN) intervals.	All the cyclic components responsible for variability in the period of the recording.
SDANN	The standard deviation of the average NN intervals calculated over 5 mins.	All the cyclic components responsible for variability during cycles longer than 5 mins.
RMSSD	The root of the mean squared differences of successive NN intervals.	Parasympathetic activity.
NN50 (NN50 count)	The number of successive intervals which differ by more than 50 ms.	Parasympathetic activity.
pNN50	The proportion of successive intervals which differ by more than 50 ms derived by dividing NN50 by the total number of NN counts. This may also be expressed as a percentage (pNN50%).	Parasympathetic activity.
SDNN	The standard deviation of all the normal-to-normal (NN) intervals.	All the cyclic components responsible for variability in the period of the recording.
Geometric measures.		
HRV triangular index	The integral of the density distribution divided by the maximum of the density distribution	All the cyclic components responsible for variability in the period of the recording.
TINN	The width of the base of a triangle fitted to the histogram of duration of all NN intervals.	All the cyclic components responsible for variability in the period of the recording.
Poincare Plots	A scattergram of each NN-interval of a tachogram plotted as a function of the previous NN-interval.	All the cyclic components responsible for variability in the period of the recording.
Frequency domain measures.		
Total Power	Variation in NN interval below 0.15Hz. The lower band may be altered depending on duration of recording.	All the cyclic components responsible for variability in the period of the recording.
LF	Variation in NN interval 0.04-0.15 Hz.	Sympathetic and parasympathetic activity.
HF	Variation in NN interval 0.15-0.40 Hz.	Parasympathetic activity.
LF (nu)	The proportion of spectral power from 0.04 – 0.4 Hz which is in the LF frequency calculated by $LF / (TP - (ULF+VLF))$	Sympathetic activity or sympathovagal balance.
HF (nu)	The proportion of spectral power from 0.04 – 0.4 Hz which is in the HF frequency calculated by $HF / (TP - (ULF+VLF))$	Parasympathetic activity or sympathovagal balance.

The above shows that a large number of variables may be produced from a single ECG. Although there are correlations between time and frequency domain measures, the replacement of one by the other has not been advocated (Taskforce 1996).

2.2.2. *Collection Methods.*

There are two general methodologies by which ECG traces for analysis of HRV can be obtained. These are 24-hour ambulatory recordings and short (5-15 min) resting recordings. Ambulatory recordings can be used to generate all time and frequency domain measures of HRV and although time domain analysis can be carried out on short-term recordings, these are more suited to analysis via spectral methods. The possible measures achieved from short-term recordings are limited further by the exclusion of the ULF and depending on recording length, the validity of VLF data. As mentioned previously, both methods have been shown to have significant clinical application and have been quantitatively validated as markers of autonomic function by several authors.

2.3. **Rejection of studies.**

All studies in Table 2-2 have been rejected from this review on statistical grounds. Three studies (Hohnloser *et al.* 1992; Burger *et al.* 1997; Dionne *et al.* 2002) were rejected for their utilisation of the Pearson product moment correlation. This statistic merely shows association between two variables and does not demonstrate reliability. Bland and Altman (1986) stated that the correlation coefficient, and likewise linear regression were totally inappropriate as measures of reliability. They stated that pattern recognition and imitation may be at work, perpetuating the use of the correlation coefficient. They also suggested the idea that referees should be responsible for returning for reanalysis papers with such incorrect statistics. Closer examination of a very recent paper (Dionne *et al.* 2002) published 16 years after the advice of Bland and Altman illustrates their point very well.

Dionne *et al.* (2002) investigated reliability of HRV in the pre and post-prandial state. Looking only at the data for controlled breathing, they reported test-retest correlation coefficients for LF of $r = 0.99$ and $r = 0.88$ pre and post-prandially.

Table 2-2 Studies rejected from review due to inappropriate statistical analysis.

Author and Date.	Subjects.	Data Collection and HRV parameters measured.	Statistical Analysis	Values Obtained Parameters.	Considered a Reliable Measure by Authors?
Dionne <i>et al</i> (2002)	Fourteen healthy non-obese volunteers.	Two sets of ECG s recorded for 14 – 16 min, supine with controlled breathing (12 bpm) in the latter half. Recording repeated after standard breakfast 1-2 weeks apart.	Pearson product moment correlation coefficient.	HF, $r > 0.74$ before and after breakfast with normal breathing but ($r < 0.58$ when breathing controlled. LF, $r > 0.72$ before and after breakfast, with controlled breathing $r = .99$ and $.88$ before and after breakfast.	LF is reliable under controlled breathing conditions. LF is reliable under non-controlled breathing conditions
Burger <i>et al.</i> (1997)	Twenty three Type 1 diabetics.	Four 24-hour ambulatory ECGs made. Time and frequency domain measures taken at baseline, 3,6 and 9 months.	Pearson product moment correlation coefficient. Analysis of variance.	Time domain 0.35 – 0.98, rMSSD was most reliable. Frequency domain 0.61 – 0.93. Not significantly different after 3, 6, 9 or 12 months. VLF and LF were most reliable.	Yes, they claimed it to be excellent.
Madas <i>et al</i> (1996)	Seventeen patients with coronary artery disease	Seven 24 hour ambulatory ECG recordings carried out over one month.	One way analysis of variance	Time domain analysis carried out. ANOVA P -value not significant.	Yes.
Kamalesh <i>et al</i> (1994)	Nineteen patients with chronic stable angina.	Two consecutive 24-hour ambulatory ECG recordings.	Repeated measures t -test.	No time domain variables were significantly different ($p > 0.05$). In frequency domain analysis there were no significant differences ($p > 0.05$), Total Power was close to difference ($P = 0.09$)	Yes, for both time domain and frequency domain.

Vardas <i>et al.</i> (1994)	Twenty patients with unexplained syncope.	Two sets of 2 min intervals just before tilt, just after tilt and at the end of tilt. repeated 1 – 6 weeks apart.	Repeated measures <i>t</i> -test.	Only frequency domain parameters measured. No differences in TP or HF from test one to test. Before the end of tilt LF measures differed significantly.	Yes, the lack of differences between means indicated reliability.
Gerin <i>et al.</i> (1993)	Thirty-seven healthy volunteers.	Two, controlled 24 hour ambulatory ECG recordings taken on consecutive days.	Pearson product moment correlation coefficient.	Only RMSSD used as measure of HRV. Correlation coefficients found to be modest ($r = 0.41$)	No, modest values reported to indicate poor reproducibility of HRV measures.
Hohnloser <i>et al.</i> (1992)	Seventeen healthy medical students, 13 patients with chest pain and 9 post myocardial infarction patients.	Three 24-hour ambulatory ECG recordings carried out on days 1,7 and 28 of the study.	Pearson product moment correlation	For all time domain parameters $r = 0.63 - 0.92$. For all frequency domain parameters $r = 0.79 - 0.88$. Values similar for all groups and between readings at 1, 7 and 28 days.	Yes, both time and frequency domain measures show good reliability independent of disease state and time between readings.

* Explanations of statistical tests and abbreviations used can be found in Appendix 1. All abbreviations relating to HRV measures can be found in Table 2-1.

Looking at the descriptive statistics it is possible to see that the pre-prandial means for LF are very close (test 1 = 23 345 Hz Eq and test 2 = 23 327 Hz Eq), test 1 is within 0.01% of test 2. However, if the post-prandial LF power descriptive data are analysed (test 1 = 22 045 Hz Eq and test 2 = 29 881 Hz Eq); it can be seen that tests 1 and 2 differ by 26.3%. On the basis of the strength and significance of the correlation coefficients the authors conclude that the LF measurements are reproducible. This conclusion is even more alarming when the post-prandial LF data under controlled breathing conditions are analysed. This test-retest condition had a smaller correlation coefficient ($r = 0.57$) which was close to statistical significance ($P = 0.07$). However, the mean scores for tests 1 and 2 demonstrated very well the weakness of this analysis. The mean score for test 1 was 26 264 Hz Eq and the mean for test 2 was 10 127 Hz Eq, therefore the means score on test 2 is only 38.6% of that on test 1.

The Pearson product moment correlation was also used by Hohnloser *et al.* (1992) who reported good reliability of time and frequency domain measures. (See Table 2-2). However, over the shorter, 7-day test-retest the percentage difference for HRV measures are given. For normal subjects these show poor reliability ranging from 10.7 – 23.5% and for patients with chest pain and post myocardial infarction patients from 18.4 – 20.2%.

Burger *et al* (1997) correlated time and frequency domain measures of HRV at baseline with repeated measures at 6, 9 and 12 months. The majority of correlation coefficients were $r > 0.75$, which was the criteria set by the researchers to demonstrate good reliability. In addition, a set of 10 repeated measures ANOVAs were carried out. The lack of significant variation over time due to non-significant P -values was cited as further demonstration of the reliability. The reported P -values are not statistically significant ($P = 0.88 - 0.13$). By calculating the effect size (d) for test-retest from 0 – 12 months it can be seen that the real differences in the means range from medium (0.5) to large (1.76) as classified by Cohen (1982). This is an excellent example of how the results of a well-designed study, following 23 subjects over a year can be negated by inappropriate statistical analysis.

As with the use of ANOVA in the above study, several studies have demonstrated how similarity of means or aggregate agreement has been wrongly used to assess reliability. This method does not necessarily indicate individual-subject agreement, which is a pre-requisite for reliability of measurements (Lee *et al.* 1989). Statistically, the repeated measures *t*-test is often limited in its application due to its inability to detect systematic bias. On a more simple level the repeated measures *t*-test simply indicates whether the group means are different or not, and whether this difference is significant. Kamalesh *et al.* (1995) simply reported means and standard deviations (log units) for all HRV measures. They carried out a repeated measures *t*-test and reported the *t*-value assuming a non-significant value to show reliability.

The use of repeated-measures *t*-tests guards against making errors in the interpretation of correlation coefficients such as those made by Dionne *et al.* (2002). However, it still gives no meaningful measure to assess the reliability of sets of scores. It simply states that the scores do or do not differ significantly from each other. The significance of a *t*-value generated in a repeated measures *t*-test is dependent on the degrees of freedom (df). When assessing whether two measures are reproducible, by simply analysing whether or not they are significantly different from one another, a type 2 error is more likely to be made when the df are large. When the df are small the opposite is true. Therefore, a significant difference between two means (lack of aggregate agreement) does not necessarily indicate lack of individual-subject agreement (Lee *et al.* 1989). The repeated measures *t*-test should be used in reliability studies only to check for systematic bias between the repeated measurements prior to a further statistical analysis of agreement between the two methods.

Madias *et al.* (1996) investigated the reliability of several HRV methods over five tests. Again, correlation coefficients were used. Due to the multiple comparisons made, a one-way analysis of variance was also employed to detect any differences between the recordings over time. There are several statistical problems with the analysis used in this study (Madias *et al.* 1996). If one were to use ANOVA to look at differences over time a repeated measures ANOVA should be used. A one-way ANOVA simply looks at the ratio of variance within

groups compared to between groups, whereas a repeated-measures ANOVA does this for repeated measures on the same individual. This is logically what an investigator should be interested in when assessing reliability.

Additionally, when ANOVA is used to assess reliability the same problems exist as when a repeated measures *t*-test is employed. No statistic is created to tell the researcher that the method used is reproducible, only that the test scores do not significantly differ. An additional problem is the use of the F statistic generated as an indicator of significance. This statistic depends on the variance within each set of test scores. If the spread of scores within groups is wide, the F statistic will be reduced, leading to an increased likelihood of a type 1 error being made when a reliability study is being conducted. A wide range of scores is often reported in the literature for HRV data when the test group is heterogeneous for variables such as age and gender (Reardon and Malik 1996; Sinnreich *et al.* 1998; Barnett *et al.* 1999; Kuo *et al.* 1999; Singh *et al.* 1999; Fukusaki *et al.* 2000; Fagard 2001) and disease state (Van Hoogenhuyze *et al.* 1991; Hohnloser *et al.* 1992; Bigger *et al.* 1995; Nolan *et al.* 1996; Ziegler *et al.* 1999). In these cases the use of ANOVA to assess reliability is even more limited than when the groups are homogenous.

The following questions then remain:

- a) What are the correct methods that should be used to assess reliability?
- b) When correct analysis is used, how reliable are the currently available methods to assess HRV?
- c) Is this reliability satisfactory for HRV to be used as a clinical and scientific tool?

2.4. The intraclass correlation coefficient (ICC).

The intraclass correlation coefficient (ICC) gives a directionless coefficient (R) of the agreement between two or more repeated measures ranging from 0 – 1. Published values exist giving outlines concerning what various ICC coefficients may represent in terms of a method's reliability (Landis and Koch 1977). Authors have applied differing sets of criteria. Some investigators have

supported the use of ICC (Lee *et al.* 1989; Lee 1992) whereas others have warned against its use (Bland and Altman 1986; Bland and Altman 1990). Its proponents suggest that it is an easily interpreted coefficient, which measures the agreement between two or more measures. Others (Bland and Altman 1986; Bland and Altman 1990) warn that the measure may be strongly influenced by the range of scores on each test. They state that with a heterogeneous population with a wide range of baseline scores, the R-value may be artificially inflated as it is with a traditional interclass correlation coefficient. Recommendations have also been made (Muller and Buttner 1994) as to the specific circumstances in which the ICC is or is not appropriate.

2.5. Studies using the intraclass correlation coefficient.

Numerous studies have reported very good ICCs for HRV using both ambulatory ECGs (Kleiger *et al.* 1991; Stein *et al.* 1995; Nolan *et al.* 1996; Pardo *et al.* 1996) and short-term ECG recordings (Piepoli *et al.* 1996; Pitzalis *et al.* 1996; Marks and Lightfoot 1999). Overall, the results of these studies are positive, showing good or excellent levels of reliability.

2.5.1. Ambulatory ECG recordings.

A study into the reliability of 24-hour measurement of HRV in normal subjects (Kleiger *et al.* 1991) demonstrated excellent reliability for all time domain measures ($R = 0.70 - 0.90$) and even higher ($R = 0.90 - 0.94$) for frequency domain measures over a period of 3 – 65 days.

The results from cardiac patients also suggest that HRV is stable over time. One study (Pardo *et al.* 1996) reported ICC values of $R > 0.99$ for all time and frequency domain measures. This value only represented short-term reliability as the 24-hour recordings were taken on consecutive days. In an investigation into reliability of HRV measures over a two week period, Stein *et al.* (1995) found that in the majority of time domain and all frequency domain indices of HRV, $R > 0.85$ when sampled over 24 hours. Similarly, high ICCs have been shown (Nolan *et al.* 1996) when comparing reliability of a single time domain measure

over 2-16 weeks. This occurred in normal patients ($R = 0.97$), those with ischaemic heart disease ($R = 0.94$) and diabetics ($R = 0.80 - 0.93$).

2.5.2. *Short-term ECG recordings.*

In this review, a short-term test is defined as any test that does not use 24-hour ambulatory monitoring. These tests can be subdivided into two further categories:

1. Stable, resting protocols, where the subject remains stationary during the ECG recording and a resting HR is measured
2. Test/manoeuvre protocols, where the HR response is monitored as the subject moves or performs a task during the ECG.

2.5.2.1. Stable, resting ECG recordings.

Using healthy, young volunteers Pitzalis *et al.* (1996) showed a wider range of ICC values for the frequency domain measures gained from resting short-term ECGs ($R = 0.29-0.77$). These values became more homogenous but were reduced overall when controlled respiration was used during the ECG ($R = 0.36-0.65$). The modest size of these coefficients compared with those of some of the studies mentioned below may be explained in two ways. Firstly they were calculated over three testing periods instead of two as is the case in the majority of studies. Secondly, subjects were retested over a wide range of durations from 1–265 days. This test-retest period is longer than those reported in the majority of reliability studies.

Marks and Lightfoot (1999) used a highly controlled data collection protocol to obtain stable, 2.5 and 5-min resting ECGs. They reported very high reliability in healthy subjects, demonstrated by very high ICCs. For time domain variables these values ranged from $R = 0.84 - 0.90$ and for frequency domain measures these values ranged from $R = 0.67 - 0.96$. This study again had a very small ($n=8$) sample size. Additionally the subjects were a very homogenous group of healthy, female, non-smoking subjects. Although this reduces the external validity of the paper by limiting the generalization of the study findings, it does

demonstrate that high ICC values may be found even when the population distribution is highly homogenous.

2.5.2.2. Test/manoeuvre ECG recording.

Toyry *et al* (1996) produced data incongruent with that from previous studies (Pitzalis *et al.* 1996) by demonstrating very low ICC values for time and frequency domain measures of HRV ($R = 0.03 - 0.24$) in healthy subjects over five consecutive days of tests. However, these findings should be treated with caution due to very short (1-min) ECG sampling period and the very small sample size ($n=4$). Further discussion of this paper (Toyry *et al.* 1995) can be found in the next section as reliability was also reported using other statistical analyses.

Similar to the findings in healthy subjects, good reliability of HRV measures was also demonstrated in patients with chronic heart failure (Piepoli *et al.* 1996). These researchers found similarly high ICC values ($R = 0.74 - 0.84$) to those of Marks and Lightfoot (1999) and Pitzalis *et al.* (1996) for frequency domain measures obtained from short-term recordings. These findings were obtained not only at rest but also during supine exercise at 25W, 50W and 75W and during three sequential, increasing levels of dobutamine infusion, used as two models of sympathetic activation

2.5.3. Summary of reliability assessed by intraclass correlation coefficient.

The majority of HRV repeatability studies report ICC values of $R > 0.79$ which is generally considered as excellent. On the basis of this it can be stated that reliability of time domain measures are excellent from both ambulatory and short-term ECG recordings. With the exception of the findings of Toyry *et al.* (1996) the reliability of frequency domain measures is also excellent. There appears to be only a minor reduction in reliability due to test-retest duration. Different disease states known to affect HRV show similar ICCs. Additionally,

when reviewing the ICCs for each frequency domain measure there is no single frequency that repeatedly displays poorer reliability than the others.

2.6. Reliability of HRV using coefficient of variation (CV), reliability coefficient (RC) and limits of agreement (LoG).

2.6.1. Methods.

Although the ICC has successfully demonstrated the reliability of repeated measures of HRV, a criticism of this method is that it does not give a clinically or scientifically meaningful figure by which we can judge reliability. Three methods have been used in the study of HRV reliability that give quantitative measures of how much variability there is between repeated scores. All three methods are based around analysis of the standard deviation (SD) of paired differences but express their coefficients differently. The most commonly used method is the coefficient of variation or coefficient of variability (CV). This expresses the SD of paired differences as a percentage of the population mean. The repeatability coefficient (RC) (often reported as the coefficient of repeatability) is similar but reports reliability in raw units and as 2 SDs of the differences. It further assumes that 95% of the differences observed should fall between the boundaries of the coefficient. Lastly, the limits of agreement (LoG) (Bland and Altman 1986) are calculated by the same method but is usually presented with the mean of differences (to demonstrate any systematic bias) and the ± 2 SD limits in a graphical form to allow visual inspection of the data. Visual inspection allows the reader to see systematic bias and to check the homoscedascity of the data. For a full discussion see Bland and Altman (1986).

These methods each have particular advantages and disadvantages over each other. A full account of these arguments is beyond the scope of this paper and the reader is directed to two excellent review articles on this subject (Bland and Altman 1986; Atkinson and Nevill 1998).

2.6.2. *Ambulatory recordings.*

Brief descriptions of studies investigating the reliability of HRV measures can be found in table 2-3. Huikuri *et al.* (1990) assessed only one time domain measure of HRV (SDNN) for each of 22 normal subjects. They found intrasubject CVs to range from 1 – 23% from three recordings over seven days. The interindividual CV was found to be high (24%). Zeigler *et al.* (1999) showed CVs for normal subjects to range from 4.2 – 14.8% for time domain analysis and 1.8 – 11.8% for frequency domain measures. These CVs were found to be much larger in diabetic patients, in whom reproducibility of time and frequency domain measures varied from 6.4 – 63.9% and 7.5 – 31.0% respectively. This finding is perhaps unsurprising for two reasons. First, diabetics typically display lower overall levels of HRV and second, these patients may frequently suffer a higher number of arrhythmic beats in the presence of diabetic autonomic neuropathy (Zeigler *et al.* 1999).

Kochiadakis *et al.* (1997) investigated healthy volunteers along with a group of patients with unexplained syncope. The time domain measures showed small CVs in normal subjects (1.96 – 4.74%) except for the standard deviation of the average NN intervals calculated over 5-min (SDANN) which had a CV of 36.85%. Values for reliability for the syncopal patients are not provided in an appropriate form. They are unfortunately only given as regression coefficients of day 1 and day 2 (slope and intercept values).

Using ambulatory methods in a healthy cohort of 30 children Batten *et al.* (2000) showed a very low CV (0.08 – 0.76%) for time domain measures. Although frequency domain measures showed more variation (0.04 – 2.03%), overall their reliability was shown to be excellent.

Table 2-3 Studies into the reliability of HRV measures using Ambulatory ECG data collection.

Author and Date.	Subjects.	Data Collection and HRV parameters measured.	Statistical Analysis	Values Obtained Parameters.	Considered a Reliable Measure by Authors?
Batten <i>et al.</i> (2000)	Thirty-nine 14 year old children.	Two 24-hour ambulatory ECG recordings made. Time between recordings not given.	Coefficient of Variation.	All time domain measures showed <1% CV. All frequency domain measures > 3% from 24 hour recording and > 4% from mean of 5-min recording.	Time domain and frequency domain methods are reliable for both time and frequency domain but 24-hour recordings more so.
Ziegler <i>et al.</i> (1999)	Seventeen healthy volunteers and 9 diabetic patients.	Two 24-hour Holter recordings made at a mean of 29 weeks apart.	Coefficient of Variation.	Geometric measures highly reliable (CV 1.9 – 2.5%). Time domain measures for 24-hour data on healthy subjects ranged from CV of 4.2 – 14.8% Time domain measures from 1.8% - 11.9% CV for diabetic patients significantly greater. Day/night comparisons also given.	Yes, 24-hour monitoring is reliable. More so in healthy subjects than diabetics. Results are more reliable than reflex tests (Zeigler 1992).
Kochiadakis <i>et al.</i> (1997)	Nineteen patients with >2 unexplained episodes of syncope in the last 6 months. A control group of 15 healthy volunteers.	Two consecutive 24-hour ambulatory ECGS. A passive head up tilt test was used to divide subjects into subgroups.	Linear regression and Coefficient of Variation.	Time domain analysis only. RMMSD and pNN50 most reliable, CV 2.6% AND 1.96%. SDANN was worst with CV 36.8%. Reproducibility worse in syncopal subjects.	Yes, in normal subjects a high degree of reproducibility shown. Much worse in subjects with poor reliability of HRV measures during tilt testing.

Nolan <i>et al.</i> (1996)	Twenty three normal volunteers, 67 patients with myocardial ischaemia, 87 diabetic patients.	Two 24-hour ambulatory ECGs carried out 2 weeks apart.	Repeatability Coefficient. Intraclass Correlation Coefficient.	For pNN50 counts: Normal patients, R = 0.97, RC = 0.21 Myocardial ischemia patients: R = 0.94, RC = 0.41 (log units) Diabetic patients. R = 0.41 - 0.91, RC = 0.61 - 0.98 (log units)	Yes, good (R > 0.8) for all groups and small RC mean small improvements may be of biological significance.
Pitzalis <i>et al.</i> (1996)	Twenty young, normal volunteers.	Three, 24-hour ambulatory ECG recordings made at baseline, 25 and 210 days.	Intraclass Correlation Coefficient.	Time domain analysis only: R values ranged from 0.57 SDNN - 0.79 rMSSD.	Not stated.
Pardo <i>et al.</i> (1996)	Thirty patients with stable coronary artery disease.	Two consecutive 24-hour ambulatory ECG recordings.	Intraclass Correlation Coefficient.	Time domain measures ranged from R = 0.992 for pNN50 to R = 0.99 for SDANN. Frequency domain measures were 0.99 for HF and LF.	Yes, the degree of reliability is excellent over the short time period measured.
Stein <i>et al.</i> (1995)	Seventeen patients with stable chronic heart failure.	Two 24-hour ambulatory ECG recordings carried out 2 weeks apart.	Intraclass Correlation Coefficient.	Time domain analysis ranged from SDNN, R=0.91 - pNN50 (%) R=0.27. Frequency domain analysis results were all R>0.86.	Yes, for time domain, certain variables are reliable. All frequency domain variables are.
Kautzner <i>et al.</i> (1995)	Thirty-three survivors of myocardial infarction.	A single 48-hour ambulatory ECG recording.	Comparison of the absolute values of relative errors.	Time domain analysis. Day to day errors ranged from 10 - 20% in all measures except pNN50 which showed error of 45%.	Yes, most measures were reliable on a day-to-day basis except pNN50.

Zuanetti <i>et al.</i> (1991)	Forty-nine patients with frequent ventricular arrhythmia.	Two 24-hour ambulatory ECG recordings carried out a median of 192 days apart.	Correlation. Coefficient of Repeatability	pNN50 was only measure used. There was a correlation of $r = 0.83$ between recordings. CR in log units was 0.03 ± 0.66 .	Yes, the 0.03 value indicates little bias between the two readings and the CR value shows good reproducibility.
Kleiger <i>et al.</i> (1991)	Fourteen normal volunteers.	Two 24-hour ambulatory ECG recordings carried 3 – 65 days apart.	Intraclass Correlation Coefficient. Standard Error Measurement.	All standard time domain parameters had high correlation coefficients ($R = 0.70 - 0.90$). All frequency domain parameters showed higher values ($R = 0.90 - 0.94$)	Yes the high R - values show excellent reproducibility regardless of the length between test and retest.
Huikuri <i>et al.</i> (1990)	Twenty two normal volunteers.	Three 24-hour ambulatory ECG recordings on two consecutive days and after 1 week.	Coefficient of Variation	Only 24-hour average HRV variability measured, CV 6% between days.	Yes, the mean 24-hour heart rate variability was reliable within subjects.

2.6.3. *Resting/Stable ECG monitoring.*

Freed *et al.* (1994) investigated the immediate reliability of frequency domain HRV measures in anaesthetised patients undergoing surgery. By comparing sequential 10-min periods of ECG data they reported CVs for total spectral power (TP), HF and LF of 9, 11 and 15% during a baseline measure before surgery. Further CVs of 6, 9 and 12% were reported for these variables ten hours after surgery. This suggests that frequency domain measures of HRV can be reproducible when they are derived from adjacent 10-min sampling periods. The clinical usefulness of these data is not great due to the short test-retest period. It does, however, serve to demonstrate the possible application of stable, short-term HRV measures in the frequency domain during tests such as orthostatic manoeuvre or head-up tilt.

Ponikowski *et al.* (1996) investigated the optimal length of recording for the evaluation of both time and frequency domain HRV measures drawing attention to the VLF component and its possible time dependency. Using a group of chronic heart failure patients, resting ECGs were recorded over periods of 5, 10, 20 and 40-min. The reliability of measurements made within the sampling period was analysed using both the CV and RC. Bland-Altman plots were also used to illustrate the time domain analyses. The results of this study are lengthy and the reader is directed to the original paper for a full review (Ponikowski *et al.* 1996). In brief, time domain analyses were found to show poor reliability. Reliability of the standard deviation of normal-to-normal intervals) SDNN (reported as SDRR) was relatively independent of sampling duration, CVs were 26.2, 30.2 25.3, 25.4% for 40, 20, 10 and 5-min recordings respectively. Both the percentage of sequential normal-to-normal intervals differing by 50% (pNN50%) and the logarithmic transformation of this variable showed very poor reliability, with CVs ranging from 70.2 - 139% over the same time periods. Frequency domain analysis, when expressed in raw units was very poor for all sampling durations. Coefficients of variation were very high for all measures; for TP they were

Table 2-4 Studies into the reliability of HRV measures using short-term ECG collection methodology.

Author and Date.	Subjects.	Data Collection and HRV parameters measured.	Statistical Analysis	Values Obtained Parameters.	Considered a Reliable Measure by Authors?
(Lord <i>et al.</i> 2001)	Twenty-one healthy volunteers and twenty one cardiac transplant patients.	Two 10- min ECG recordings made with subjects laid supine and paced breathing made one week apart.	Coefficient of Variation	Only data for LF analysed. CV was 45% for controls CV was 76% for transplant patients	No, CV accounted for 14 and 15% of the total population variation in each case.
(Jauregui-Renaud <i>et al.</i> 2001)	Twenty healthy volunteers.	Two 4-hour, Holter ECGs made during paced breathing, sitting, standing, and cold pressor test Measures made 2 weeks apart.	Repeatability Coefficient.	HF = 23.6 and 40.8 ms ² .Hz during paced respiration and supine to seated. LF/HF ratio 5.1 and 1.63 ms².Hz during seated to standing and supine to seated respectively.	Yes for both measures.
(Parati <i>et al.</i> 2001)	Eight hypertensive females.	Two 15 min ECG recordings during 15 min of supine rest with both spontaneous and paced taken 1 month apart.	Coefficient of Variation	All values given for pulse interval variability. For spontaneous breathing, VLF LF and HF CVs were 6, 5 and 12%. For paced breathing CVs were all 6%.	Yes for all measures.
(Salo <i>et al.</i> 1999)	Fifteen hypertensive patients with sleep apnoea and 9 age-matched controls.	Four measures taken during 2, 15 min recording: spontaneous and normal breathing, orthostatic manoeuvre and cold pressor test. Measures taken 44 weeks apart.	Coefficient of Variation	Time domain analysis: RMSSD, CV RMSSD and CVS all showed CV 21% - 57.9% in controls. Much worse in patients. Frequency domain measures were mostly > 30% but more dependent on the conditions, also better in controls than patients.	Reproducibility unsatisfactory during cold pressor and orthostatic manoeuvre tests. In other conditions said to be acceptable but only <30%.
(Marks and Lightfoot 1999)	Eight physically active, college age females, all non-smokers.	Two sets of 5 and 2.5-min ECG recordings made with subjects laid supine with paced breathing (10-12 BPM) made within 1 week.	Intraclass Correlation Coefficient	Time domain measures between day variation from R=0.67 to 0.9 Frequency domain measures between day variation from R=0.67 to 0.96. Different results for 2.5 vs. 5-min recordings R>0.67 except LF (R = 0.53)	Yes nearly all correlation coefficients classified as 'Good' or 'Almost perfect'.

(Sinnreich <i>et al.</i> 1998)	Seventy healthy Kibbutzim	Two recordings of 6-min of supine rest both with and without controlled breathing made 6-7 months apart.	Coefficient of Variation.	Figures given are for normal breathing. Time domain analysis: SDNN=6% RMSSD = 8%. Frequency domain analysis. VLF= 9%, LF= 11.5% HF =12% TP= 7%. Similar results for controlled breathing.	Yes, said to indicate considerable temporal stability.
(Cloarec-Blanchard <i>et al.</i> 1997)	Fourteen healthy volunteers.	Two recordings of 30-min rest followed by nitroglycerin infusion or 20 second tilt from supine to 60 degrees head up with 10 min ECG made 1 week apart.	Limits of Agreement. Repeatability Coefficient.	LF reported only. RC of 201 b.min ⁻¹ .Hz ^{1/2} for nitro glycerine infusion and 295 b.min ⁻¹ /Hz ^{1/2} for head up tilt. LF/HF ratio, RC 120 b.min ⁻¹ .Hz ^{1/2} . Limits were much narrower when expressed in nu.	Yes, the short term change in LF and LF/HF ratio during sympathetic activation is repeatable in healthy adult males.
(Pitzalis <i>et al.</i> 1996)	Twenty young, normal volunteers.	Three, 10-min ECG recordings during: rest, controlled breathing and passive 70 degree head up tilt. made at baseline, 25 and 210 days.	Intraclass Correlation Coefficient.	Time domain analysis: R values ranged from 0.27– 0.56. In frequency domain analysis R = 0.29 – 0.77 at baseline, 0.36 – 0.65 during controlled breathing and tilt test. LF/HF was always lowest.	Reproducibility was described as either low or fair.
(Ponikowski <i>et al.</i> 1996)	Sixteen patients with chronic heart failure.	Two 60-min recordings taken from which, 5, 10, 20, and 40 min samples extracted a mean of 25 days apart.	Reliability Coefficient. Coefficient of Variability.	Time domain analysis. Using VC, SDRR values ranged from 25.3 – 30.2% PNN50 values CVs were 100%. Duration did not affect reproducibility. Frequency domain CV values ranged from 45 – 111%. Log transformed CV values ranged from 8 – 64.1% Square root transformed VC values ranged from 24.1 – 93.5%.	Both time domain and frequency domain measures show poor reproducibility in patients with chronic heart failure.
(Piepoli <i>et al.</i> 1996)	Ten patients with chronic heart failure	Four series of 5-min stages from rest to 80% HRmax from dobutamine infusion and four stages from rest to 75 W of cycle ergometry.	Intraclass Correlation Coefficient. Coefficient of Variation.	SD was the only time domain parameter measured, VC 16%. In the frequency domain LF varied from CV 6.8% - 13.7%. HF varied from CV 14.1% - 25%	No, this study extends previous findings that HRV variables obtained from short-term static sampling periods are unreliable.

(Bootsma <i>et al.</i> 1996)	Nineteen healthy men.	Two incremental tilts from 0-80 degrees with 5-min ECGs taken every 5 degrees 1-8 months apart.	Coefficient of Variation	Slopes of regression lines for LF% and tilt analysed. CV was 22% for all subjects.	No, large intersubject and intrasubject variation in slope of lines.
(Toyry <i>et al.</i> 1995)	Four healthy men.	Five tests repeated on five successive days; controlled breathing, deep breathing Valasalva manoeuvre, baroreflex sensitivity test and orthostatic test. One min of ECG data analysed	Intraclass Correlation Coefficient and Coefficient of Variation.	Time domain analysis. RMMSD CV was 18%, R = 0.24 The frequency domain parameters CVs were 28 – 52%, R = -0.03 – 0.24.	There was no general trend toward increase or decrease and therefore no learning effect. The day-to-day reproducibility was poor.
(Freed <i>et al.</i> 1994)	Fifteen patients undergoing surgery via either anaesthetic or epidural.	Two sets of adjacent 10-min segments of ECG analysed during pre- and post operative periods.	Coefficient of Variation.	Pre-operative CVs for frequency domain analysis; TP 9%, HF 11% and LF 15%. Post operative CVs for frequency domain analysis: TP 6%, HF 9% and LF 12%.	Immediate variability in frequency domain HRV variables is low.
(Ziegler <i>et al.</i> 1992)	Twenty healthy subjects and twenty-one type 1 diabetics.	Varying data collection times during, lying supine, standing, deep breathing, and Valsalva manoeuvre on consecutive days.	Intrasubject standard deviation factor (SDF)	Time domain parameters CV and RMSSD showed Sd_{intra} values of 1.26 and 1.46 at rest. They were reduced to 1.18 and 1.27 during deep breathing. Frequency domain parameters ranged from 1.5 – 1.71 at rest. Values similar in diabetics.	Reproducibility of HRV parameters not discussed.

45.9-57%, for VLF, 3.6-53.4%, for LF, 79.5-111.7% and for HF, 66.1 - 71.4%. The authors applied both square root and log transformation to the data in an attempt to reduce these coefficients. The latter was more successful, reducing the CVs for TP and VLF to <10% and HF to 19.9 - 22.3%. The CV for LF power remained at an unacceptably high level (32 - 64%). Following log transformation, it was clearly shown that the reliability of LF is positively related to the duration of the sampling period. The authors conclude that the reliability of HRV measures, especially pNN50 and LF is very poor in chronic heart failure patients.

Reliability of resting HRV in a large group (n=70) of healthy subjects was also investigated by Sinnreich *et al.* (1998). These investigators reported CVs for frequency domain measures from 5-min recordings taken in the field of 6.1 – 12.0% and 6.0 -10.7% for free and metronomic breathing respectively. These CVs from 5-min duration ECG recordings taken six to seven months apart are significantly lower than those reported by Ponikowski *et al.* (1996) for 5, 10, 20 or 40 min recordings for frequency domain measures.

A further study investigated the reliability of the LF component of resting HRV in healthy patients cardiac transplant recipients (Lord *et al.* 2001). These researchers found poor agreement between measurements made a week apart. CVs were large for normal subjects (45%) and transplant recipients (76%). It was concluded that the LF component of HRV was not reproducible, especially in cardiac transplant recipients. It should be noted, however, that very little RR interval variability is present in the denervated heart of a transplant recipient. Such low values are prone to large test retest CVs due to only very small test-retest differences.

2.6.4. *Test/manoeuvre ECG recording.*

Cloarec-Blanchard *et al.* (1997) investigated the reliability of change in HRV due to sympathetic activation via nitroglycerin infusion and 60° head-up tilt on healthy subjects. The researchers used $\text{beats}\cdot\text{min}^{-1}\text{Hz}^{-1/2}$ to report their frequency domain

units as opposed to the recommended msec^2 . Using Bland-Altman plots and RC it was found that from test 1 to test 2 there was no systematic bias and showed limits of agreement of ± 201 and $0.039 \text{ beats}\cdot\text{min}^{-1}\text{Hz}^{-1/2}$ for raw and normalised units respectively during nitroglycerin infusion. The limits for 60° head-up tilt were slightly larger at ± 347 and $0.072 \text{ beats}\cdot\text{min}^{-1}\text{Hz}^{-1/2}$ for raw and normalised units respectively. These limits were deemed to provide sufficient precision for the method to be used over the test-retest period (one week). An important point addressed was the length of the recording. The short (5 min) recording periods used in this study were defended on the grounds of their increased likelihood of being stable measurements and in being useful for assessing HRV and changes in HRV during a series of short tests. The authors also constructed sample size tables. These tables clearly show the use of normalised units to be preferential in terms of reducing sample size in a study using such a measure. Further discussion of these tables may be found later in this paper when the necessary level of reliability is addressed. The use of 5-min recordings is also deemed to be preferable when examining the changes in HRV during a given period such as the cold pressor or orthostatic tolerance test

Toyry *et al.* (1995) used a battery of tests (deep breathing, Valsalva manoeuvre, phenylephrine infusion and an orthostatic test) to investigate baroreflex function in healthy subjects. From all the tests the mean CV reported for the root mean square differences of normal to normal intervals (RMSSD) was 18%. For frequency domain measures CVs were poor, ranging from 28 - 39%). The results of this study should be observed with caution due to the short and varied duration of testing (40 s to 5 min) and in the very small subjects numbers ($n = 4$).

Salo *et al.* (1999) also compared reliability in healthy subjects and patients with hypertensive sleep apnoea during a battery of tests including, spontaneous breathing, deep breathing, orthostatic manoeuvre and cold pressor test. Reliability of RMSSD was measured by CV and found to be relatively poor throughout the conditions in both normal subjects (19.0 – 48.0%) and in hypertensive sleep apnoea patients (30.4

-57.9%). In normal subjects, the repeatability of LF varied greatly but was mostly very poor with values from 3.0 - 46.6%. For HF similar values (3.0 - 87.5%) were found as was true for TP. Reliability of the LF:HF ratio was even worse with almost all values greater than 34.0%. Overall, reliability was significantly worse for all frequency domain measures in the patients with hypertensive sleep apnoea (3.3 - 197.8%). It was concluded that time domain analysis was more reproducible than frequency domain methods (Salo *et al.* 1999). It was clearly stated that some of the very high CVs found were not acceptable but it was also stated that some coefficients, namely those <30% were acceptable. The basis of such a cut off point is not explained. The consequence of creating such a point will be discussed later when the necessary level of reliability is addressed.

Bootsma *et al.* (1996) examined the reliability of %LF during repeated head up tilt in healthy subjects. They specifically examined the reliability of the slope of the regression line between increased tilt and increase in %LF. Using the CV it was found that the slopes of these lines varied by 22% between test and retest.

Only one study has made comparisons between reliability of HRV measures in healthy subjects and patients (diabetics) during test manoeuvres (Ziegler *et al.* 1992). Using the Standard Deviation Factor (SDF), it was found that diabetic and healthy subjects showed similar levels of reliability for the time domain measure of RMSSD and the CV or RR intervals (1.2 - 1.3%). Frequency domain variables showed poorer reliability in both controls (1.5 - 1.7%) and in diabetics (1.4 - 1.7%). This is the only study in the published literature found to use this method of analysis and the authors justification, although sound, is beyond the scope of this paper. For comparison with other studies the SDF can be expressed as variation in percent by the following formula: $(SDF-1) * 100 \%$. It can be seen that the variation shown between tests in this study is high for both time (18 - 34%) and frequency (37 - 71%) domain measures of HRV.

Jauregui-Renaud *et al.* (2001) used short-term ECG analysis to study healthy subjects undergoing a variety of tests. They reported the reliability coefficient (RC) but called it the 95% coefficient of reliability and unusually reported it as a percentage where it is normally expressed in raw units. This percentage can be considered as approximately twice the CV. In all frequency domain variables reported, 95% or more of cases fell within the RC. These limits were however found to be rather large. For HF power expressed as normalised units (HFnu) they were 40.8, 23.6, 28.6 and 37.9% respectively during the following conditions: paced breathing, supine to seated, seated to standing and cold pressor test. For the same battery of tests the RC values for LF:HF ratio were much smaller at 3.6, 1.6, 5.1 and 2.8% respectively.

2.7. Discussion of Findings.

2.7.1. Introduction.

Prior to any discussion of the findings of the above studies it is necessary to indicate the complexity of analysing such a data set. The Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology Report (Taskforce 1996) lists 18 possible measures of HRV which can be measured using either 24-hour ambulatory or short-term stable conditions. Add to this the large number of possible study populations such as healthy individuals, diabetics, CHF patients and the number of possible outcomes is large. Vary the duration over which reliability is assessed from sequential readings through to a year as is shown in the literature reviewed here and the number of possible options runs into the hundreds. Within the short-term recordings there are a number of 'tests' which have been employed such as cold pressor test, orthostatic maneuvers and the number of possible reliability coefficients runs into the thousands. This is of course ignoring the different statistical techniques employed to demonstrate reliability.

Instead of systematically reviewing the effect of each of these variables while attempting to hold the remainder constant, the following discussion will focus on trends displayed in the data. This will be done by posing a number of pertinent questions and attempting to answer them using evidence from the literature. Results from the literature concerning these trends may not always be completely homogenous. Where this occurs attempts to explain differences in findings will be made.

2.7.2. *Ambulatory vs. short-term tests – which are more reliable?*

The results of studies using ICC suggest that 24-hour Holter tape monitoring of ambulatory ECG data to be a more reproducible method of HRV analysis than short-term recording of stable ECGs. It should be noted, however, that both methods have demonstrated excellent levels of reliability for HRV using the ICC. When other statistical methods are employed to assess reliability the results have not always been in agreement. Overall there is much more variation in reliability coefficients when they are reported using statistical methods other than ICC. In general it can be seen that CVs are larger for both time and frequency domain measures when short-term ECG data are analysed (Ponikowski *et al.* 1996; Lord *et al.* 2001) although some values reported still show good reliability (Freed *et al.* 1994; Sinnreich *et al.* 1998). Both ambulatory and resting methods have large reported ranges of reliability values. Reliability values for ambulatory recordings have been reported as poor (Kochiadakis *et al.* 1997; Batten *et al.* 2000) and there is significant overlap between the coefficients for ambulatory and resting methods (Huikuri *et al.* 1990; Ziegler *et al.* 1992) . From this evidence it is not possible to state categorically whether one method of data collection yields consistently more reliable results compared with the other.

For clinicians and researchers alike it is encouraging that short-term and ambulatory tests have been shown to possess similar reliability coefficients. This may increase the use of HRV as a regular screening tool for cardiac patients, especially post-MI

patients and it may be employed as a fast, non-invasive method to increase the accuracy of risk assessment in these populations. Although ambulatory HRV data have shown to be independently predictive of cardiac events in a healthy elderly cohort (Tsuji *et al.* 1994; Tsuji *et al.* 1996), patients with stable, chronic angina (van Boven *et al.* 1998; Weber *et al.* 1999; Forslund *et al.* 2002) and in post-MI patients (Kleiger *et al.* 1991; Bigger *et al.* 1993; Forslund *et al.* 2002). Such a relationship has only recently been demonstrated using short-term recordings (Sosnowski *et al.* 2002). It should be noted that the 5-min recording in this study was actually extracted from a 24-hour Holter recording after being identified as the first stable 5-min period free of ectopic beats. Further studies that have demonstrated short-term methods to be reliable (Sinnreich *et al.* 1998) may allow quantification of the predictive power of these easily undertaken methods.

From a practical stand-point this means that results from the different methods cannot yet be used interchangeably. The two methods measure different aspects of HRV. Certain measurements in the time domain should not be generated from short term recordings (Taskforce 1996). Certain frequency domain measures cannot be calculated accurately from short (5 min) sampling periods. Oscillations in HR in the Very Low Frequency band (VLF) have been proposed as a marker of sympathetic activity (Saul *et al.* 1988). Even slower changes in the Ultra Low Frequency band (ULF) are also known to exist (Kobayashi and Musha 1982) which as yet required clarification as to their physiological significance. According to the recommendations of the Taskforce (1996), neither of these measures can be calculated accurately from short (5 min) ECG recording periods.

The two types of data collection methods have different sources of error that contribute to their overall reliability. The main source of variation from 24-hour recordings is the activity of the subject during monitoring, including physical activity, psychological stress and other factors known to affect HR. In short-term recordings the subject is also the biggest source of variation. What is of primary importance is whether the reading is a truly stable ECG and changes in data

collected are more likely to be from antecedent variables as opposed to changes during the test period itself. It is then surprising how broadly similar the various measures of reliability are for both of these methods.

One pattern that emerges is that short-term assessment of HRV, when combined with a battery of tests, seems to yield less reproducible results than stable short-term tests and ambulatory data. In addition to this, more complex tests seem to yield consecutively less reproducible results compared with more simple ones. The Taskforce. (1996) recommend 24-hour ambulatory data collection and/or 2-5 min stable collection techniques. It may be that HRV measures in the frequency domain (which are those which can be accurately assessed in short sampling periods) may not be suited to measuring HR response during manoeuvres. More traditional responses to these bedside tests such as HR and blood pressure have been shown to be more reliable when assessed concurrently with HRV (Toyry *et al.* 1995).

2.7.3. *Time domain vs. frequency domain analysis – which is more reliable?*

Ambulatory studies using the ICC which report values for both time and frequency domain measures of HRV are scarce. From those that have been carried out, there is no definitive answer to the above question. Frequency domain measures from 24-hour recordings have been shown to be more reliable than time domain (Kleiger *et al.* 1991) or at least equal to them (Stein *et al.* 1995; Nolan *et al.* 1996; Pardo *et al.* 1996). Using the CV to measure reliability, Zeigler *et al.* (1999) showed time and frequency domain measures to be equally reliable whereas Batten *et al.* (1999) demonstrated frequency domain measures to have lower overall reliability than frequency domain measures. It should be noted that all CVs in this study were found to be excellent and the poorest value still only demonstrated 2% variation. Where both time and frequency domain measures have been calculated from short-term stable ECG recordings, the results also indicate similar levels of reliability. However the level of reliability between studies is not consistent and both excellent (Marks

and Lightfoot 1999) and very poor (Pitzalis *et al.* 1996) levels of reliability have been reported.

What these studies demonstrate is that when ambulatory recordings are used there seems to be no difference in the reliability between the time or frequency domain analyses of ECG data. Although findings are somewhat inconsistent, overall no large discrepancies seem to occur. Knowledge of this fact and the high degree of intercorrelation between certain time and frequency domain measures (Bigger *et al.* 1992) may be helpful in enabling researchers to choose which variables they investigate and report. By reducing the often large number of colinear variables reported in investigations into HRV, the statistical analysis and the description of findings may be simplified.

One clear pattern that emerges in the literature is the reduced reliability of studies that use manoeuvres or bedside tests. The amount of variation between tests is almost uniformly >10% and commonly around 30% (Toyry *et al.* 1995; Cloarec-Blanchard *et al.* 1997; Salo *et al.* 1999). Although good reliability of certain variables has been shown (Jauregui-Renaud *et al.* 2001) this seems to be the exception rather than the rule.

2.7.4. *The effect of disease state of study population on reliability.*

The 1996 Task Force recommended that HRV should only be used in two clinical situations: as a predictor of risk after acute MI and as an early warning sign of diabetic neuropathy. Despite this, levels of HRV have been assessed in numerous populations and coefficients for the reliability of HRV in these populations are available.

Using the ICC, excellent reliability has been shown in healthy patients (Kleiger *et al.* 1991) and in cardiac patients (Pardo *et al.* 1996). Nolan *et al.* (1996) also found similar ICC values for healthy volunteers, ischaemic heart disease patients and

diabetics. However, Nolan *et al.* (1996) also used the RC to show reliability. Looking at these statistics there is a clear increase in the size of the RC from healthy subjects (0.21 log units) to ischaemic heart disease patients (0.41 log units) to diabetics (0.61-0.87 log units). It should be noted however that for each group the time from test to retest was progressively longer.

Using a battery of tests, Salo *et al.* (1996) found greatly decreased reliability in patients with hypertensive sleep apnoea compared with healthy controls. Poor reliability of HRV was shown by Ponikowski *et al.* (1996) in chronic heart failure patients, whereas Sinnreich *et al.* (1998) showed very good reliability of short-term measures when healthy subjects were studied. Zeigler *et al.* (1999) found CVs approximately three times larger when assessing reliability of HRV in diabetic patients in comparison with healthy controls. Lord *et al.* (2001) showed a very high CV for normal subjects (45%), which was significantly increased in heart transplant recipients (76%).

It seems that healthy subjects produce more reliable HRV results compared to a number of populations studied. This would seem to suggest that caution is needed when a test is used in a population with a specific condition unless the method itself had been shown to be reliable in that population. Generalisation from studies which have shown a test to be reliable in a normal population may be inappropriate for a group in a specific disease state. This is especially true when reliability coefficients are being used to calculate sample sizes for investigations. A power calculation based on the reliability coefficient gained from a normal healthy population may lead to the underestimation of necessary sample size and reduced statistical power in the study of an alternative population.

2.7.5. *The effect of time from test to retest on reliability.*

The evidence discussed here suggests reliability is independent of the time from test to retest. Although some of the most reliable measurements have been generated

from sequential ECG data (Pardo *et al.* 1996) but when the interval between recordings is 24-hours or more, there seems to be little effect of retest duration on reliability. Comparison of like with like in terms of duration is very difficult. This is partially due to the large spread of test-retest durations and the large number of other variables. Overall, although some short test-retest periods demonstrate very good reliability (Kochiadakis *et al.* 1997) others do not (Kautzner *et al.* 1995). Values for reliability have also been shown to be very similar from one test to another regardless of whether the test was over a long duration (Sinnreich *et al.* 1998) or over two sequential readings (Freed *et al.* 1994). In one study (Nolan *et al.* 1996) four groups of diabetic patients were retested at varying periods. The RC (log units) values showed an effect of time on reliability as the coefficients increase with time with values of 0.61 (29 days), 0.68 (63 days) 0.98 (92 days), and 0.87 (115 days). These observations indicate that the test-retest duration and reliability relationship is not linear.

2.7.6. *Do certain measures demonstrate lower reliability than others?*

In the analysis of time domain measures there are no clear patterns. The variables listed in each analysis are numerous and varied. It would seem that due to their interdependence on each other, poor reliability on one variable is often accompanied by poor reliability on another. Kautzner *et al.* (1995) and Stein *et al.* (1995) reported anomalous values for pNN50 and PNN50% respectively when compared to other measures of HRV. Kautzner *et al.* (1995) recognised that pNN50 may be an inferior measure in the population tested (acute MI survivors). Similarly, the greatly reduced ICC value in the study of Stein *et al.* (1995) was attributed to the low levels of pNN50% in the population studied (congestive heart failure patients). These findings may be of importance when choosing time domain analyses for such a population.

The literature is equivocal as to whether one frequency domain measure is more or less reliable than its counterparts. Several studies demonstrate LF power to possess poorer reliability than other spectral components (Freed *et al.* 1994; Piepoli *et al.*

1996; Sinnreich *et al.* 1998) whereas other studies show the least reproducible variable is HF power (Marks and Lightfoot 1999; Jauregui-Renaud *et al.* 2001).

2.7.7. *The appropriate levels of reported accuracy.*

Within the literature the criteria for categorisation of the various statistics used to demonstrate reliability of methods are varied. For instance using the ICC, some researchers Pitzalis *et al.* (1996) cite the categories put forward by Landis and Koch (1977). Others (Nolan *et al.* 1996) cite alternative categories (Bartko 1966). When the CV has been used, the description of an acceptable amount of error has varied from 5% (Lord *et al.* 2001) based on recommendations found in the Task Force Report (1996) to a seemingly arbitrary 30% put forward without justification (Salo *et al.* 1999). Atkinson and Neville (1998) advise caution when such seemingly arbitrary analytical goals such as the commonly adopted target for a CV less than 10% are adopted.

Instead of applying such rigid assessment criteria, researchers should make qualitative decisions as to whether the accuracy of their measurement is suitable for the proposed use. To this end the RC and LoG are excellent methods by which to assess reliability. The latter displays the upper and lower-most limits of a biologically significant change in the variable of interest Cloarec-Blanchard *et al.* (1997) calculated the RC and LoG of LF and LF:HF ratio during head up tilt and nitroglycerin infusion. During the more commonly used test of sympathetic stimulation of head up tilt they found an RC of $201 \text{ beats}\cdot\text{min}^{-1}/\text{Hz}^{-1/2}$. Using the variances estimated in the study, they calculated the necessary sample size to ensure sufficient statistical power in future studies. They did not however base the differences expected on any empirical data and instead simply gave a range of sample sizes for detecting expected changes from $25 \text{ beats}\cdot\text{min}^{-1}/\text{Hz}^{-1/2}$ to $250 \text{ beats}\cdot\text{min}^{-1}/\text{Hz}^{-1/2}$.

Although this is of some use for future research, the application of such criteria is limited by the data from which they are derived. Again, using Cloarec-Blanchard *et al.* (1997) as an example, their findings are limited methodologically to research projects concerning changes in the LF and LF:HF components of HRV during head-up tilt. The applications of these findings are also limited by the population on which the original study was carried out, in this case, healthy adult males. No expected values due to tilt are cited by the authors thereby limiting the usefulness of the sample size calculations given. Without being over-critical of the work of Cloarec-Blanchard *et al.* (1997) it is obvious that if one were to study a group in which the usefulness of the head-up tilt technique has been demonstrated such as syncopal patients (Fitzpatrick *et al.* 1991; Rubin *et al.* 1993), the sample sizes cannot be accurately estimated from the tables provided. This is because the necessary sample size is derived in part from the effect size (d). When assessing the impact of measurement reliability, it is necessary to modulate d to create the Measurement Error Correction (Hunter and Schmidt 1990). Where RC is reported, the MEC is obtained by: $MEC = d\sqrt{RC}$. From the literature there is some, albeit limited, data to suggest that reliability of this manoeuvre is slightly worse in syncopal patients than healthy controls (Vardas *et al.* 1994). This would increase the value of RC and eventually decrease the statistical power of any study using the measure. Ironically this study (Vardas *et al.* 1994) is one of those rejected from the original discussion of reliability due to inappropriate statistical analysis. Vardas *et al.* (1994) used t -tests to determine reliability, a statistic which cannot be used to compute the MEC.

2.8. Conclusions and Recommendations.

From the studies reviewed, it is possible to make several recommendations. All studies into the reliability of HRV measures should use appropriate statistical analyses. The following should clearly be excluded: the Pearson product moment correlation, repeated measures t -tests and repeated measures ANOVA (unless being used in conjunction with another method to assess reliability).

Appropriate statistics may include the ICC, although caution as to which model is employed should be taken. Many of the studies cited in this review give no details of how the ICC was calculated or which of the six possible models of ICC were used.

The coefficient of variation may also be used. This statistic should be accompanied by a check for systematic bias and a check for homoscedascity of the data, neither of which occur by the calculation of CV.

The British Standards Institution recommends the use of the RC. This coefficient is best used in conjunction with the LoG which can easily be calculated from the RC. Again, a check for systematic bias should be carried out. The LoG then checks the homoscedascity of the data.

On the basis of this review the following method for establishing suitable levels of reliability has is recommended and has been adopted for current and future research.

1. The intended reliability of the method should be made *a priori* on the basis of previous research.
2. This should include an estimate of sample size based on:
 - a. Expected effect size based on previous empirical data or pilot data
 - b. An estimate of reliability of the measurement either from the literature but preferably from empirical investigation.
3. Using the approach advocated by Hunter (1990) the necessary sample size for the desired statistical power should be calculated.
4. If the sample size is logistically and financially viable, the research should go ahead as planned.
5. If the sample size is too great two approaches are open to the researcher:
 - a. a smaller sample is used and the reduced statistical power of the study is reported.
 - b. the researcher must attempt to improve the reliability of the method.

Using the above process (1-4), the investigator can then determine whether the degree of reliability displayed by the method is acceptable for their own purposes. Furthermore, due to the large variation in previously reported values for reliability on HRV measures all investigators should be encouraged to publish their own coefficients.

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CHAPTER 3. AGREEMENT AND RELIABILITY OF THREE DIFFERENT METHODS USED TO ASSESS HEART RATE VARIABILITY.

Abstract.

Numerous instruments are commercially available to measure heart rate variability yet little is known concerning the agreement between such instruments. The objective of the present study was to assess agreement between measures of heart rate variability in three commercially available instruments.

Thirty subjects (20 males) median age 27.5 (range 19-59 years) underwent simultaneous ECG recordings, under three different resting conditions: supine, standing and supine with controlled breathing, using three commercially available analysers. Intraclass correlation coefficients showed excellent agreement (lower 95% C.I, $R > 0.75$) between all instruments under all conditions. ANOVA revealed small but significant ($P < 0.05$) differences in SDNN in all conditions and RR interval length recorded in the standing and controlled breathing conditions. This was due to discrepant ECG recording protocols that were unrelated to consistent operator timing.

This study demonstrates that measures of HRV generated by the three instruments agree well. Any discrepancies were due to the recording protocols of the systems. This may lead to incomparable results between instruments. It is therefore recommended that: a) if different instruments are used in the same study or b) multi-center study designs are planned or c) heart rate variability results are discussed with reference to studies using other instruments, levels of agreement need to be reported to ensure comparability.

Different aspects of this chapter, in truncated form, have been published in *Clinical Physiology and Functional Imaging and Physiological Measurement*, see appendix VI.

3.1. Introduction

Experimental and clinical studies have shown heart rate variability (HRV) to be a measure of autonomic modulation (Akselrod *et al.* 1981; Pagani *et al.* 1988; Malliani *et al.* 1991; Pagani *et al.* 1997) and particularly cardiac vagal tone (Hohnloser *et al.* 1992; Badra *et al.* 2001; Goldberger *et al.* 2001). There is much interest in the use of HRV as a non-invasive measure of autonomic control. This is partially due to the fact that decreased HRV is an independent indicator of sudden cardiac death in patients following acute myocardial infarction (Kleiger *et al.* 1991) and in the general population (Tsuji *et al.* 1996).

There are two distinct methodologies that can be used in the assessment of HRV. The majority of studies have used 24-hour, Holter recorded, ambulatory ECG collection. From such recordings, time and frequency domain analyses of the whole data set are carried out. Such long-term recordings are not always practical or feasible and it has been shown that useful, predictive measures can be gained from shorter ECG recordings (2 – 15-min) either extracted from the longer, 24-hour Holter recordings (Bigger *et al.* 1993; Faber *et al.* 1996) or from analysis of a single ECG recorded under stable, usually resting, conditions.

In 1996 the European and North American Task Force report on measurement of HRV (Taskforce 1996) recommended that either method could be used and gave specific guidelines for the type of analysis that should be carried out on the data gained from short and long-term recordings. The report recommends that frequency domain measures may be easier to interpret in terms of physiological regulation than time domain measures and that they be carried out in preference to time domain analysis in short (2 – 7-min) recording periods. It was also recommended that a standard duration of 5-min be used for comparison between studies.

Data from 24-hour ambulatory recordings have shown attenuated HRV in a variety of disease states such as angina (Weber *et al.* 1999; Forslund *et al.* 2002) heart failure (Saul *et al.* 1988; Scalvini *et al.* 1998) diabetes (Ewing *et al.* 1991),

following myocardial infarction (Kleiger *et al.* 1991; Bigger *et al.* 1993) and in a variety of other cardiovascular conditions (Huikuri *et al.* 1999; Yamada *et al.* 2000; Hayano *et al.* 2001) . Data from short-term recordings are more limited and HRV has been shown to be attenuated in only a few disease states, such as hypertension (Konrady *et al.* 2001) and following myocardial infarction (Bigger *et al.* 1993; Faber *et al.* 1996)

In epidemiological studies using healthy populations, short-term recordings have been more extensively utilised. In such studies, indices of HRV show variation according to age, sex and lifestyle (Byrne *et al.* 1996; Gregoire *et al.* 1996; Fukusaki *et al.* 2000; Kuch *et al.* 2001; Migliaro *et al.* 2001) in a manner similar to those of 24-hour ambulatory recordings (Coumel *et al.* 1994; Reardon and Malik 1996; Stein *et al.* 2000) .

From the distribution of dates in the literature it is clear that short-term HRV measurements are becoming more commonplace. Numerous laboratories are utilising new technologies to make large numbers of faster, short-term HRV recordings. In order to confirm global findings and ensure comparability between laboratories using HRV measurements it is important to quantify the agreement between the methods of assessment being used. There are a large number of commercially available instruments capable of measuring HRV (Kennedy 1995). Systematic bias between instruments used in different studies would make comparison of the results of those studies difficult. Systematic bias between instrumentation used in a single, multi-centre study, for example, would invalidate the findings unless the bias had been reported and corrected either methodologically or statistically. In either case, knowledge of the agreement between instruments is of great value. Therefore, whenever more than one instrument is used in data collection it is recommended that a suitable coefficient to describe agreement be reported by the researchers.

3.1.1. Potential sources of error in HRV analysis.

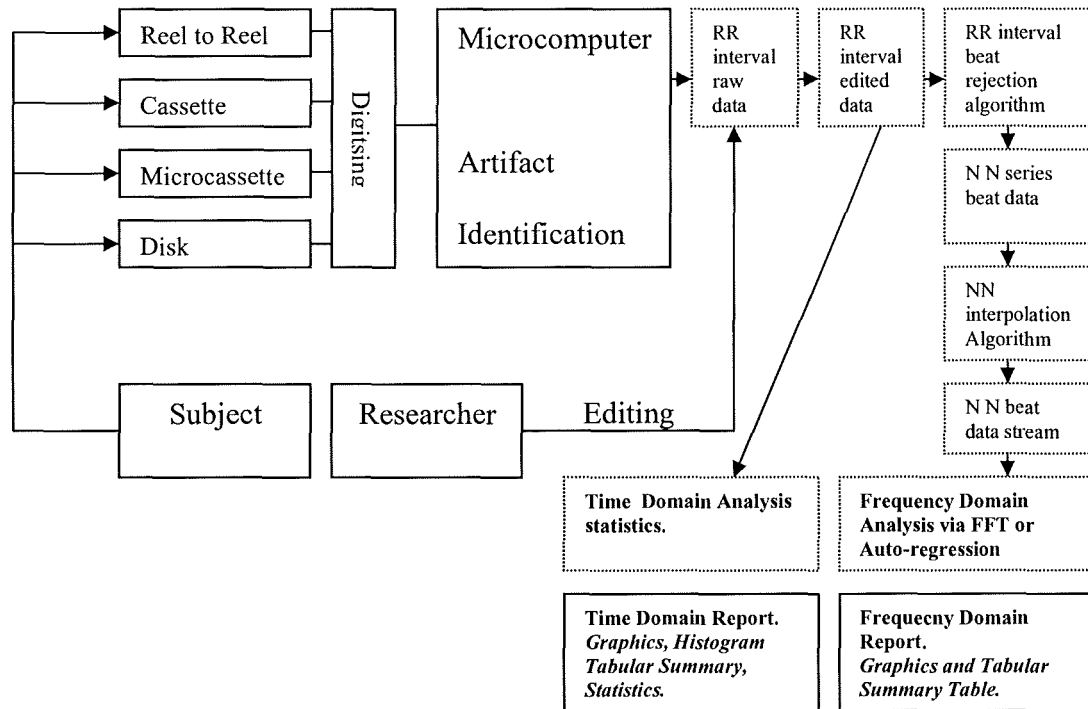


Figure 3-1 The components of HRV instrumentation (Adapted from Kennedy 1995).

Kennedy (1995) provides a schematic representation of the instrumentation involved in the process of HRV analysis. Figure 3-1 has been adapted from this and illustrates points at which variability in data collection, analysis and reporting may appear. By employing such a model and substituting the specifications of more than one set of HRV instrumentation it is possible to identify points where variation in measurement and analysis may occur.

Ideally, if one were to monitor and analyse a single ECG trace (or electronic signal) using various pieces of HRV instrumentation, each would produce identical results. If this is not the case then the cause of the variation should be investigated and identified. Once identified it may be possible to eradicate intra-equipment variation. One benefit of using a simulated ECG signal or trace with known characteristics is that an accurate measure of the internal validity of the equipment can be gained. The

known input should produce a known output and any variation from the expected output is due to instrumentation. One disadvantage is that the external validity of the instrumentation is not tested. Any error from ECG recording, storing or digitisation may not be assessed. More importantly the researcher gains knowledge concerning the instrument's ability to measure a real subject's HRV.

A variety of methods have been used to assess agreement or concurrent validity between instruments. What follows is a brief review of the available literature concerning variation in measurement and analysis of HRV. There is a paucity of data related directly to this topic for two reasons:

- a) The volume of literature comparing any HRV methods is very limited.
- b) Where this literature exists it mainly concerns analysis of 24-hour ambulatory data.

Whereas many of the issues relating to analysis of 24-hour data are shared with those of short-term recordings, others are clearly of less importance in short-term HRV analysis. To provide a full review, all threats to inter-equipment validity will be discussed. Due to the scarcity of pertinent literature, those studies mentioned will be described and examined in detail.

3.1.2. Studies assessing agreement between HRV instrumentation.

Molgaard (1991) examined the concurrent validity of the Reynolds Pathfinder 2 system (Reynolds Medical, Hertford, UK) by examining the agreement between HRV measures derived from simulated ECG signals on this system and the Oxford Medilog 4-24 (Reynolds Medical, Hertford, UK). Very small coefficients of variation (CVs) were demonstrated for time domain HRV measures between the two systems. Where variation did occur it was said to be due to detection of 'false long NN pauses' (normal-to-normal beat intervals) or extended RR intervals. These were said to be due to movement of the recording tape. Although this study (Molgaard 1991) is an example of the assessment of agreement between two commercially available methods to measure HRV the methodology elicits little insight into

possible sources of variation in short term measurements such as those addressed in the present study.

In a second such study (Jung *et al.* 1996) agreement of HRV measures derived from eleven Holter tapes recorded on a single Holter recorder (Tracker, Reynolds Medical, Hertford, UK) analysed on four commercially available HRV analysis systems :

- a) Del Mar Avionics HRV Analyzer Version 1.01, Irvine, California;
- b) Ela Medical Version V1.27, Montrouge, France;
- c) Marquette Version 5.8, Milwaukee, Wisconsin;
- d) Oxford Instruments HRV Version 7.11, Abingdon, United Kingdom.

Analysis of differences in mean recordings from the systems by ANOVA showed that time domain measures from the various systems were not comparable. Although ANOVA does not strictly assess agreement the large differences in means for each system suggest the level of agreement would be unacceptable between the systems. *Post-hoc* analyses of the means suggested good agreement between the Oxford Instruments HRV Version 7.11 and the Ela Medical Version V1.27 system on certain variables but not all.

It was concluded that Holter tape analysis using different commercially available systems is statistically not comparable. One reason put forward for this was differences in the removal of ectopic beats although this could not be confirmed due to the insufficient provision of visual information by all the systems. There was no relationship between HRV variables and the number of beats sampled or number of beats removed.

A critical appraisal of the study by its authors concluded that the use of a tape recorded on one system, being analysed by another system led to the poor measures of agreement. This was described as an inherent weakness of the study. The authors

noted the attractiveness of repeating such a study utilising a methodology involving the simultaneous application of four different Holter recorders, each corresponding to the HRV systems to be used for subsequent analysis. It was concluded that this was not feasible at the time of the study.

An approximation of the above proposal was undertaken two years later (Simula *et al.* 1998). Simula *et al.* (1998) made simultaneous, 24-hour ECG recordings using analogue (Marquette 8500, Milwaukee, Wisconsin) and digital (Oxford Medilog FD-3, Hertford, UK) recorders. The digital signal was analysed using an Excel Medilog 2 system. The analogue signal was firstly analysed using the software in the Marquette system and again, using the Excel system. The authors stated that HRV analysis followed the 'careful' removal of ectopic beats but gave no details of how this was undertaken. Two separate comparisons were made in the study.

- a) The comparison of the digitally derived HRV parameters (spectral and temporal) with those derived from an analogue signal
- b) The comparison of an analogue ECG signal after processing by a digital recorder.

The former pair of variables was found to show excellent agreement. The latter pair showed poor agreement and significant bias. This demonstrated, similarly to Jung *et al.* (1996), the problems associated with analysing analogue ECG recordings using a digital analyser.

What the above studies (Jung *et al.* 1996; Simula *et al.* 1998) illustrate is that when Holter recorded ECG traces are fed into their original or designated analysis software the systems show agreement in terms of the HRV measurements produced. There are further possible sources of variation not addressed fully in these studies, namely those that do not come from the analysis system itself.

One such source of variation is observer annotation of the ECG signal generated from the Holter tape. Kroll *et al.* (1996) investigated the observer reliability regarding rhythm annotation in the analysis of ambulatory HRV and the effect of

interobserver differences in calculating temporal and spectral measures. Eleven ECGs from nine patients with varying amounts of both atrial and ventricular ectopy were analysed on the same HRV analysis system (Marquette Laser XP, Milwaukee, Wisconsin) by two different physicians. The repeatability coefficient (RC) was determined as the square root of the sum of the squares of the differences between paired values from each trial divided by the sample size. This is equivalent to the standard deviation of the paired differences. The coefficient of variability (CV) was determined as the standard deviation of the paired differences divided by the mean of each set of measurements was also calculated. The low-frequency measures such as the standard deviation of the mean for all normal to normal intervals in each 5-min epoch (SDANN) demonstrated the lowest (0.6%) CVs. Measures of higher frequency oscillation, such as the number of intervals differing by more than 50 milliseconds (pNN50) or the root mean square of the standard deviation of adjacent intervals (RMSSD) had CVs more than ten times the variation of the low frequency measures (7.7% and 7.5% respectively).

Due to consistency in the equipment used, the source of variation demonstrated in this study (Kroll *et al.* 1996) was identified as originating from interobserver differences in beat annotation. Indeed it was found that the interobserver differences in calculated measures of high frequency HRV were correlated with differences in annotation of supraventricular ectopy. There was no relationship between interobserver annotation and low frequency HRV variables. The latter showed excellent reliability in any case.

There was no interobserver disagreement in the presence or absence of ventricular ectopy, and therefore little effect on the calculated high frequency variables. Between 50% and 75% of the variation observed in high frequency variables was explained by interobserver differences in identification of supraventricular ectopy and sinus arrhythmia.

The multi-centre study design has frequently been the chosen method for HRV studies to obtain large sample sizes and to utilise pre-recorded Holter tape recordings for subsequent analysis of HRV. In such a design there are numerous combinations of recorders, analysers and investigators which may all contribute to the final presentation of HRV data. Therefore, the effect of data analysis in different laboratories on HRV measures needs to be quantified. Yi *et al.* (2000) studied 24-hour ambulatory recordings from post myocardial infarction (MI) patients using either a Reynolds Tracker Recorder (Hertford, UK), (R) or a Marquette 8500 system (Milwaukee, Wisconsin, USA) (M). The same tapes were then analysed using three different analysis systems at three venues by four independent operators.

At centre one, the M and R were both used. Full visual reviewing of M took place but R was used with minimal intervention, beats were rejected by being 63% lower or 175% higher than preceding NN intervals. The tapes were also analysed at a second centre with a CardioData Mk4 analysis system (Mortara Instruments inc., Milwaukee, Wisconsin, USA) which uses template matching for ectopic beat removal. In this analysis, operator over-reading and interpretation were also used. The data were finally reanalysed at a third centre, again using the CardioData MK4 (using same strategy as before) but analysed by an operator with no knowledge of previous readings. Only time domain parameters were calculated and all were global readings, except the short-term measure of RMSSD.

Unlike the findings of Jung *et al.* (1996) NN interval was found to be highly reliable, as were all global readings of HRV. The agreement between systems in the assessment of RMSSD was found to be poor. Due to the large percentage of the total measurement represented by the limits of agreement it was concluded that, particularly in post-MI patients, consistent classification of RMSSD may be difficult to achieve in a multi-centre study.

The intersystem agreement reported in this study (Yi *et al.* 2000) is actually a combination of interobserver error and technical system error. With the exception of

RMSSD the cross-instrument analyses were shown to be less problematic than the combinations used by Jung *et al.* (1996). Additionally, the technicians were all highly trained in the analysis and editing of Holter tapes, and the errors resulting from their analyses were found to be less than those reported previously by Kroll *et al.* (1998). These data seem to suggest that high levels of agreement may be achieved in a multi-centre study using different analysis systems when adequate care is taken within all aspects of the analysis.

Data concerning the agreement of HRV parameters assessed during short-term recordings of heart rate is limited to a single study (Carrasco *et al.* 1998). Carrasco *et al.* (1998) assessed agreement between four spectral and four temporal HRV parameters obtained from ECG and blood pressure wave analysis in 10 healthy subjects under 5 different conditions.

Blood pressure wave was recorded using a Finapres monitor, which gives numerical details of heart rate, systolic and diastolic blood pressure using a photoplethysmograph as a servo to a blood pressure cuff. The cuff can then respond to maintain a reference value allowing monitoring of the blood pressure. This allows the measurement of the arterial pressure wave (APW) which is in turn digitised. This digitised signal is then sent to a computer via a communication protocol of the manufacturer's design. The ECG signal was obtained through a CM5 lead from a 78330A ECG monitor which was band-pass filtered (1.0-250 Hz) and damped (-40 dB/dec). This signal was A/D converted and digitised at 500 Hz prior to analysis.

Both the ECG signal (RR interval data) and the APW signal (PP interval data) were filtered and interpolated similarly in accordance with the Task Force recommendations (Taskforce 1996) using the R wave fiducial point and consecutive maxima of the pressure wave as measures of heart rate and blood pressure respectively.

Agreement was assessed by analysis of means, correlation and limits of agreement. Strangely the limits provided were 2.26 SD either side of the mean difference not 1.96 as recommended (Bland and Altman 1986). Although some of the temporal parameters measured displayed good limits of agreement in resting measures, there was a systematic underestimation of these HRV variables by the Finapres monitor especially during standing and exercise conditions. A similar pattern of results was shown in the frequency domain analyses except in the standing condition where the PP data significantly overestimated total power (TP), high frequency power (HF) and underestimated low frequency power (LF) and the ratio of low to high frequency power ratio (LF:HF). From these results it was concluded that the two methods were not interchangeable.

From the available data it therefore, seems viable to hypothesise that when simultaneous measures of ECG activity are made on a subject and subsequently analysed by their designated HRV analysis system high levels of agreement between the methods should be evident. This hypothesis is based mostly on data from 24-hour Holter tape analyses. Whether this translates to short-term recordings of the ECG is unknown. From the data provided by Carrasco *et al.* (1998) it seems that short-term recordings show some inter-equipment agreement under certain conditions. Utilising simultaneous ECG recordings as opposed to blood pressure wave should serve to enhance agreement between commercially available methods to determine HRV.

3.1.3. *Studies assessing reliability of HRV instruments.*

The Task Force report noted that there were limited data on the reliability of HRV measures over time. Good reliability of HRV measures is important if changes in HRV due to interventions are to be observed. Of equal importance to researchers and clinicians is the comparability of different methodologies used to assess HRV in different laboratories.

Numerous investigations have been carried out to assess the reliability of HRV measures using ambulatory, (usually 24-hour) ECG recordings (Huikuri *et al.* 1990; Kleiger *et al.* 1991; Van Hoogenhuyze *et al.* 1991; Zuanetti *et al.* 1991; Durant *et al.* 1992; Ziegler *et al.* 1992; Klingenheben *et al.* 1993; Kamalesh *et al.* 1995; Kautzner *et al.* 1995; Stein *et al.* 1995) Investigations into the reliability of short-term measures of HRV are less numerous but have become more commonplace recently. These studies can be subdivided into those for which the ECG has been recorded under resting or stationary conditions (Freed *et al.* 1994; Piepoli *et al.* 1996; Ponikowski *et al.* 1996; Sinnreich *et al.* 1998) (Marks and Lightfoot 1999; Jauregui-Renaud *et al.* 2001; Lord *et al.* 2001) and those which investigate change in HRV measures during some form of test or manoeuvre (Ziegler *et al.* 1992; Ponikowski *et al.* 1996; Sinnreich *et al.* 1998; Jauregui-Renaud *et al.* 2001; Lord *et al.* 2001) . The results of both these groups of investigations vary widely due, in part to the varying statistical analyses used and also to the different populations studied.

The increase in research into short-term HRV analysis in the frequency domain is undoubtedly connected with recent advances in data collection and automated processing through custom-built software. Time and especially frequency domain analysis of short term ECG data has, therefore, become simpler and more accessible. This does not, however, ensure results are comparable. In fact, a greater reliance on automated data analysis by custom-built software results in more opportunities for differential treatment of ECG data. This may in turn, result in greater variation in the analysis and results between laboratories.

The aim of the present investigation was twofold. First, to determine if simultaneous ECG measurements analysed by three commercially available HRV instruments yielded similar results in terms of the HRV parameters produced. The second aim was, to determine whether the results from each instrument were stable over time. The first part of this study is, therefore, an assessment of the concurrent validity of three instruments. The second part is an assessment of their absolute reliability as

defined by Baumgartner (1989). Both of these constructs are of great importance when undertaking empirical research.

3.2. Methods.

3.2.1. Study population.

All procedures were approved by the local ethics committee. All volunteers gave full written informed consent for their participation in the study. Thirty healthy volunteers (20 males) with a median age 27.5 (range 19-59 years) agreed to take part in the study. All subjects indicated they were in good health as defined as absence of cardiovascular disease and were not on any medication which may have affected HRV during the study period.

3.2.2. Instrumentation and data analysis.

Heart rate variability was measured simultaneously using three automated pieces of equipment: the HVR analysis module within the CardioPerfect ST software using the Medical Graphics CardO₂ CP stress system (*Medical Graphics Corporation, St Paul, Minnesota USA*), a Cardiotens 24-hour ambulatory ECG recorder (*Meditech Ltd. Budapest, Hungary*) and a TF5 HRV analysis instrument (*Advanced Medical Diagnostics Ltd., Leeds, UK*). Table 3-1 gives the technical specifications of the equipment used.

3.2.3. Study design.

Subjects attended the laboratory on two occasions a mean of seven (SD \pm 3) days apart. On each occasion subjects underwent three simultaneous measurements of HRV. Subjects were asked to refrain from strenuous exercise and heavy alcohol consumption on the day prior to the test and to abstain from caffeine containing foods and beverages and tobacco use on the day of the tests. Subjects were

instructed to eat a light breakfast at least two hours prior to testing. All testing was conducted between 8.00 AM and 1.00 PM.

Table 3-1 Technical specifications of the three HRV instruments used.

Name	Recording Method	Sampling Rate	Analysis	Frequency bands	RR interval filtering and interpolation	Measures Given
Medgraphics Cardio Perfect HRV Module (CP)	12-Lead ECG directly into non-portable recorder	1000 Hz	Fast Fourier Transformation or Discrete Fourier Transformation	Fully variable. Default values: VLF 0.0033-0.04 LF 0.04-0.15, HF 0.15-0.40	Automated and manual filtering available	Total Power, VLF, LF, HF in raw and normalised units. All Time-domain
CardioTens Ambulatory ECG recorder. (CT)	5-Lead ECG and portable recorder.	500Hz for ECG 1000Hz for RR intervals	Fast Fourier Transformation	Non-variable. LF 0.04 – 0.15 HF 0.15 – 0.40	Automated filtering only.	Total Power, LF and HF. LF:HF ratio in raw and normalised units.
VariaCardio TF5 HRV analyser. (TF5)	Chest strap with signal to remote wireless receiver.	500 Hz for ECG 1000 Hz for RR interval.	Fast Fourier Transformation	Non-variable. LF 0.04-0.15 HF 0.15 – 0.40	Automated and manual filtering available.	Total Power, VLF, LF and HF in raw and normalised units SDNN and MSSD.

For a full list of abbreviations and definitions of terms see Appendix 2.

On reporting to the laboratory subjects were prepared for the three recordings. This included the cleaning and preparation of the skin for the attachment of surface electrodes (*Medicotest Ltd, Denmark*). Due to the simultaneous recordings, placement of electrodes for the 12 lead ECG was slightly modified. Lead V4 was placed slightly lateral to the midclavicular line and V5 was placed slightly medial to the anterior axillary line. All five-lead ECG electrode placements were standard and the TF5 chest strap was correctly placed in accordance with manufacturer's instructions.

Subjects were then asked to lie supine on a bed while the signals from the equipment were checked for interference and signal quality. This allowed the subject's HR to stabilise. The time taken for this was at least five minutes.

Each subject then underwent three sequential 5-min ECG recordings under the following conditions.

- 1 Lying supine with natural breathing (supine)
- 2 Standing with natural breathing (standing)
- 3 Lying supine with controlled respiration at 12 breaths per min (breathing)

For conditions 1 and 2 subjects lay or stood in a quiet laboratory and were instructed to relax but remain wakeful. For condition 3 subjects were also asked to breathe in time with a metronome with verbal instructions to breathe in and out in a rhythmic manner which elicited a speed of 12 breaths•min⁻¹. Between each condition subjects were given at least 3 minutes to adjust to change in posture and to practise the controlled breathing exercise. During this time the ECG was monitored, but these recordings were not entered into the analysis of HRV.

3.2.4. *Heart rate variability analysis*

Both the CP system and the CT automatic protocols were set to sample 300 seconds of ECG recording. The automatic protocol in the TF5 system sampled either 300 seconds or 300 beats whichever was the longer. Following recording of the ECG data the three systems were set to edit out artefacts using their default automated protocols. For both the TF5 and the CT system the default criteria for the removal of an abnormal beat was an RR interval differing by >200 ms from the previous one. For the CP system the default value was a difference of 10% between beats. After removal of abnormal beats, both the TF5 and CT systems performed their analysis on 256 NN intervals. The CP system linearly interpolated NN intervals based on preceding intervals replacing any abnormal sections of data with interpolated beats. The CP system allowed a full beat-by-beat visual inspection of the length of each RR interval and of the raw ECG signal. The TF5 allowed inspection of the length of each RR interval via a graphical representation. These latter features allowed for manual removal of abnormal beats but this mode was not used in the present study. Instead, for consistency, only automatic filtering was used for all three systems.

The second part of the data analysis was the transformation of the NN interval data into frequency domain data. This was again carried out by the default method. In all three systems this was fast Fourier transformation. The frequency domain data was then reported in the frequency bandwidths recommended in the Task force report (Camm *et al.* 1996): VLF, (0.0033-0.04 Hz) LF (0.04 – 0.15 Hz) and HF (0.15 – 0.40 Hz). These frequencies were again, the default values for each system. Only HF, LF in raw and normalised units and LF:HF ratio were used from the frequency domain analysis. Measures of TP could not be compared between all three instruments as the CT and TF5 report TP as LF+HF (0.04-0.4Hz) whereas TP reported by the CP also includes the VLF band (0.0033 – 0.4Hz) Additionally, TP was not compared as a measure due to the short (5-min) recording duration. It has

previously been stated that the values of TP and VLF may be dubious when obtained from such short recordings and should be avoided (Taskforce 1996).

The Taskforce also recommend that RMSSD and SDNN be the only time domain measures taken from stationary, 5-min recordings. On this basis, only these measures were extracted from the time domain analysis produced by the three instruments.

3.2.5. *Statistical analysis.*

All statistical analysis was carried out using SPSS version 11.0 (SPSS inc. Chicago, Illinois, USA). Prior to statistical analysis, the normality of all data was assessed using a Kolmogorov-Smirnov test.

To assess agreement between the three instruments the intraclass correlation coefficient (ICC) was calculated between the three data sets. A number of possible coefficients may be calculated to assess agreement. A mixed model 3.1 ICC is recommended for use when more than two trials are being assessed (Hopkins 2000). This ICC is unbiased for any sample size. Both the R-value for the ICC and the upper and lower 95% confidence intervals (CI) were reported in accordance with previous recommendations. A lower 95% C.I. of $R = 0.75$ was interpreted as demonstrating methods to show close enough agreement to be able to replace one another (Lee *et al.* 1989; Lee 1992). Lee *et al.* (1989, 1992) and others (Bland and Altman 1986; Bland and Altman 1990) have recommend the use of a test of mean differences in addition to the ICC. A test which generates no probability value is recommended, such as a plot of mean values versus test-retest differences. The use of more than two data sets necessitated the use of a repeated-measures ANOVA to avoid multiple plots. Alpha was not adjusted for multiple comparisons because in this case, the experimental hypothesis is that no difference between the means exists.

Adjustment of alpha in this scenario would increase the likelihood of making a type one error.

To assess absolute reliability the coefficient of variation (CV) was calculated. This statistic is the standard deviation of the mean squares of pairs of values divided by the mean of the population and expressed as a percentage. This allows easy interpretation as well as permitting the researcher or clinician to make a value judgement about the suitability of the method. The CV can be used in the calculation of sample size for future research using numerous formulae. This fact, and the use of this statistic in previous studies prompted the choice of CV from the array of reliability statistics available. Additionally, as the CV does not utilise an alpha value it is suitable for the multiple comparisons in the present study.

For global comparison with previous data as recommended by (Atkinson and Nevill 1998) the ICC 3.1 was also calculated for pairs of repeated measurements to estimate reliability from each instrument. This model has been previously recommended for reliability analysis (Shrout and Fleiss 1979).

3.3. Results.

Data were gathered from 30 volunteers. Data from one participant were not entered into the analysis due to the detection of a suspected arrhythmia. This participant was referred to their general practitioner for further testing according to guidelines set out by the local ethics committee. The retest data on a second subject was rejected after analysis due to anomalous decrements in all HRV parameters. It was found that this subject had consumed a considerable amount of alcohol the previous evening. Standing data on one subject were omitted due to the occurrence of a cartilage injury between test and retest and technical failure lead to the omission of controlled breathing data for another subject.

3.3.1. Agreement.

To assess agreement, rm-ANOVA and ICC were performed on the data obtained from the first test, 28 complete data sets were entered into the analysis. Tables 3-2 – 3-4 show the mean (\pm SD) HRV values from simultaneous ECG measurements by the three instruments in conditions 1-3, the values of repeated measures ANOVA as test of systematic bias and ICCs with 95% C.I. as a measure of agreement.

All frequency domain parameters showed high levels of agreement as indicated by the high values of the ICC. None of these values showed any systematic bias between the means for measurements made by each instrument (rm-ANOVA, $P > 0.05$). Although excellent agreement between the time domain parameters was shown, mean values of time domain parameters were not as homogenous. Repeated measures ANOVA demonstrated systematic bias approaching statistical significance ($P = 0.06$) for RMSSD and significant bias ($P = 0.05$) for SDNN and RR interval data between machines. *Post-hoc* analysis by paired *t*-test showed that in both cases, the overall increase in variance between groups was due to a significant difference existing in measurements from the CT instrument.

In condition 2 (standing), high levels of agreement between instruments were demonstrated in the majority of frequency domain measures. One parameter which was an exception to this was the LF:HF ratio in which the lower bound of the 95% C.I. for the ICC was less than the recommended level of $R=0.75$ to show excellent agreement. In the lying position, rm-ANOVA and *post-hoc* paired *t*-tests demonstrated the time domain parameter SDNN to again be systematically overestimated by the CT instrument.

Table 3-2. Mean (\pm SD) scores and reliability of all instruments in data collection condition 1:lying supine with free breathing.

HRV Measure	TF5	CardioTens	Cardioperfect	ANOVA	ICC	95% C.I
LF (ms ²)	918.4 \pm 986.5	961.7 \pm 1002.4	987.4 \pm 1213.6	$P=$.36	.97	.94-.99
LF (nu)	45.5 \pm 23.8	47.2 \pm 22.0	46.1 \pm 24.5	$P=$.60	.95	.91-.98
HF (ms ²)	1194.4 \pm 1434.4	1185.1 \pm 1323.8	1245.8 \pm 1454.6	$P=$.67	.99	.98-1.0
HF (nu)	54.5 \pm 23.8	51.1 \pm 21.8	52.4 \pm 23.1	$P=$.18	.97	.94-.98
LF:HF	1.6 \pm 2.2	1.5 \pm 2.0	1.5 \pm 1.8	$P=$.79	.95	.92-.98
RMSSD (ms)	45.7 \pm 24.9	45.7 \pm 23.6	47.9 \pm 25.3	$P=$.06	.99	.98-1.0
SDNN (ms)	53.3 \pm 20.7	63.2* \pm 25.8	54.2 \pm 20.9	$P=$.05	.93	.87-.96
Mean RR (ms)	944.0 \pm 133.8	937.6* \pm 131.2	945.7 \pm 135.8	$P=$.05	.99	.99-1.0

LF – low frequency spectral power, HF – high frequency spectral power, nu – normalised units, LF:HF – the ratio of low to high frequency spectral power, RMSSD – route mean square of the standard deviation of normal to normal interval differences, SDNN – the standard deviation of normal to normal intervals, mean RR – mean time (ms) between normal r-waves.

* Indicates result is significantly ($P < 0.05$) different from both other instruments.

Table 3-3. Mean (\pm SD) scores and reliability of all instruments in data collection condition 2:standing with free breathing.

HRV Measure	TF5	CardioTens	Cardioperfect	ANOVA	ICC	CI
LF (ms ²)	1396.2 \pm 1921.8	1635.8 \pm 1952.7	1485.4 \pm 1663.0	$P=.45$.95	.90-.97
LF (nu)	67.2 \pm 20.1	70.5 \pm 18.8	66.7 \pm 22.8	$P=.35$.92	.85-.96
HF (ms ²)	456.1 \pm 502.7	513.5 \pm 551.2	577.3 \pm 627.9	$P=.28$.92	.85-.96
HF (nu)	32.8 \pm 20.1	28.7 \pm 18.2	33.2 \pm 22.8	$P=.23$.91	.86-.93
LF:HF	3.9 \pm 4.1	5.3 \pm 8.2	4.4 \pm 5.9	$P=.38$.79	.60-.90
RMSSD (ms)	30.5 \pm 16.1	32.1 \pm 15.5	33.1 \pm 16.8	$P=.45$.95	.90-.98
SDNN (ms)	52.9 \pm 21.9	63.1* \pm 23.7	54.8 \pm 21.2	$P=.01$.96	.93-.98
Mean RR (ms)	795.3 \pm 102.7	795.4 \pm 102.9	807.4 \pm 110.2	$P=.31$.99	.97-.99

LF – low frequency spectral power, HF – high frequency spectral power, nu – normalised units, LF:HF – the ratio of low to high frequency spectral power, RMSSD – route mean square of the standard deviation of normal to normal interval differences, SDNN – the standard deviation of normal to normal intervals, mean RR – mean time (ms) between normal r-waves.

* Indicates result is significantly ($P < 0.05$) different from both other instruments.

Table 3-4: Mean (\pm SD) scores and reliability of all instruments in data collection condition 3: lying supine, controlled breathing (12 breaths.min⁻¹)

HRV Measure	TF5	CardioTens	Cardioperfect	ANOVA	ICC	CI
LF (ms ²)	966.2 \pm 1652.7	868.8 \pm 1151.1	889.3 \pm 1485.8	$P=.69$.98	.97-.99
LF (nu)	35.5 \pm 22.8	36.4 \pm 22.2	39.1 \pm 25.7	$P=.22$.98	.97-.99
HF (ms ²)	1379.6 \pm 1060.1	1505.6 \pm 1293.1	1186.4 \pm 955.8	$P=.08$.91	.83-.96
HF (nu)	64.5 \pm 22.8	62.0 \pm 21.8	61.0 \pm 25.7	$P=.28$.93	.87-.96
LF:HF	1.6 \pm 2.9	1.5 \pm 2.3	1.5 \pm 3.2	$P=.27$.88	.76-.94
RMSSD (ms)	50.0 \pm 21.2	68.4 \pm 23.0	51.4 \pm 20.2	$P=.26$.98	.97-.99
SDNN (ms)	56.0 \pm 20.5	68.7.0* \pm 28.4	55.2 \pm 18.5	$P=.001$.90	.81-.95
Mean RR (ms)	972.7* \pm 140.6	970.0* \pm 134.4	975.6* \pm 143.4	$P=.001$.99	.99-1.0

LF – low frequency spectral power, HF – high frequency spectral power, nu – normalised units, LF:HF – the ratio of low to high frequency spectral power, RMSSD – route mean square of the standard deviation of normal to normal interval differences, SDNN – the standard deviation of normal to normal intervals, mean RR – mean time (ms) between normal r-waves.

* Indicates result is significantly ($P < 0.05$) different from both other instruments.

CV (%) TF5 in all three conditions.

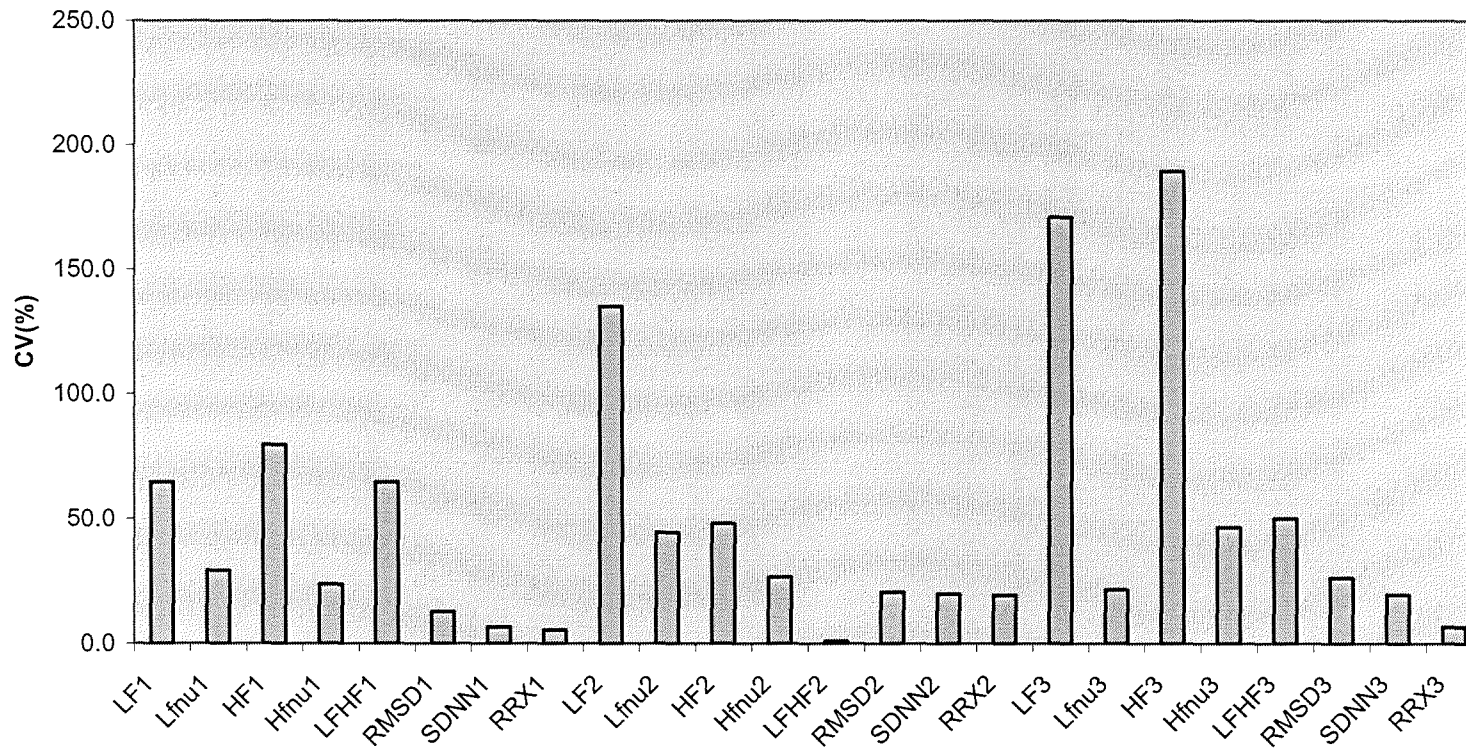


Figure 3-2: CV (%) for TF5 for all HRV measures in conditions 1-3.

LF – low frequency spectral power, HF – high frequency spectral power, nu – normalised units, LF:HF – the ratio of low to high frequency spectral power, RMSSD – route mean square of the standard deviation of normal to normal interval differences, SDNN – the standard deviation of normal to normal intervals, mean RR – mean time (ms) between normal r-waves.

* Indicates result is significantly ($P < 0.05$) different from both other instruments.

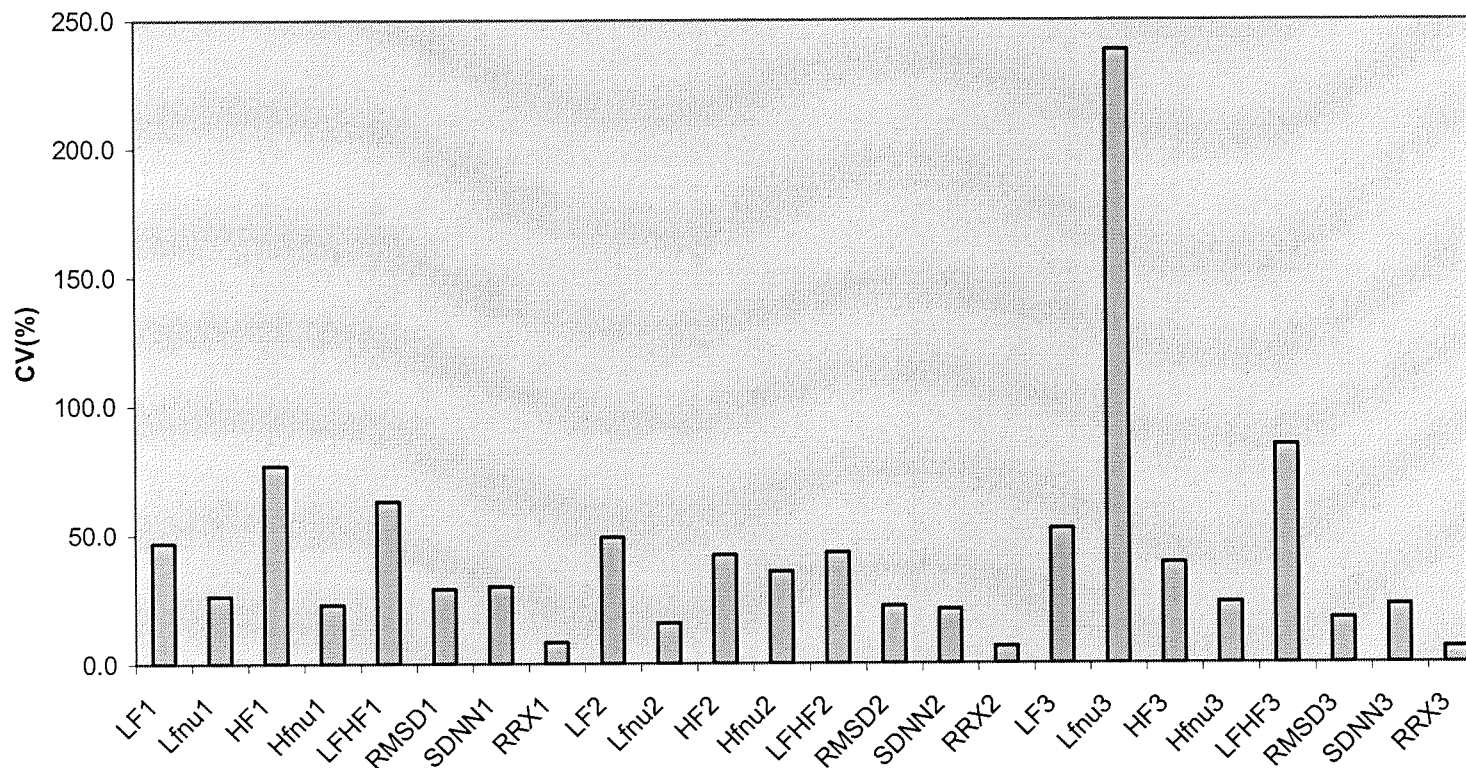


Figure 3-3 CV (%) for CT for all HRV measures in conditions 1-3.

LF – low frequency spectral power, HF – high frequency spectral power, nu – normalised units, LF:HF – the ratio of low to high frequency spectral power, RMSSD – route mean square of the standard deviation of normal to normal interval differences, SDNN – the standard deviation of normal to normal intervals, mean RR – mean time (ms) between normal r-waves.

* Indicates result is significantly ($P < 0.05$) different from both other instruments.

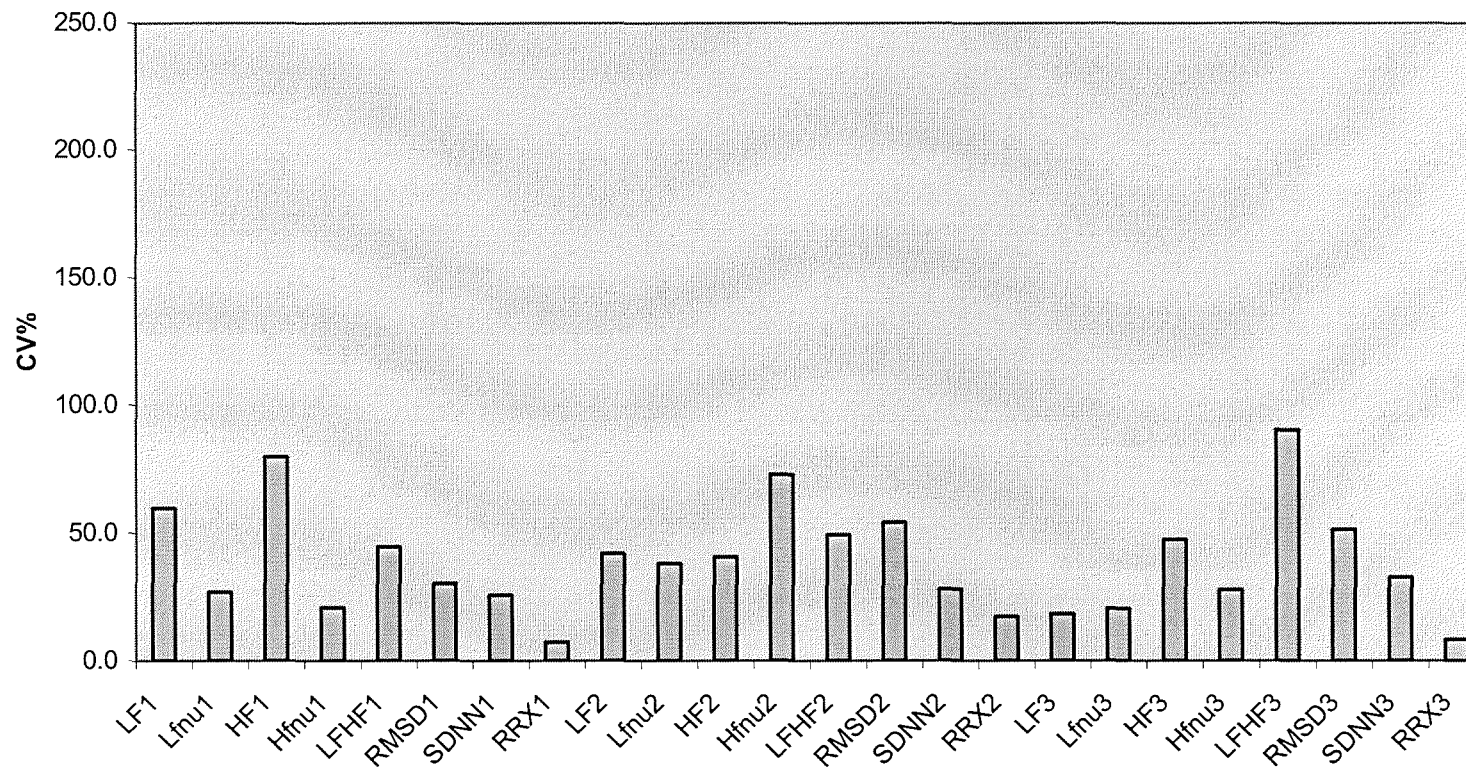


Figure 3-4. CV (%) for CP for all HRV measures in conditions 1-3.

LF – low frequency spectral power, HF – high frequency spectral power, nu – normalised units, LF:HF – the ratio of low to high frequency spectral power, RMSSD – route mean square of the standard deviation of normal to normal interval differences, SDNN – the standard deviation of normal to normal intervals, mean RR – mean time (ms) between normal r-waves.

* Indicates result is significantly ($P < 0.05$) different from both other instruments.

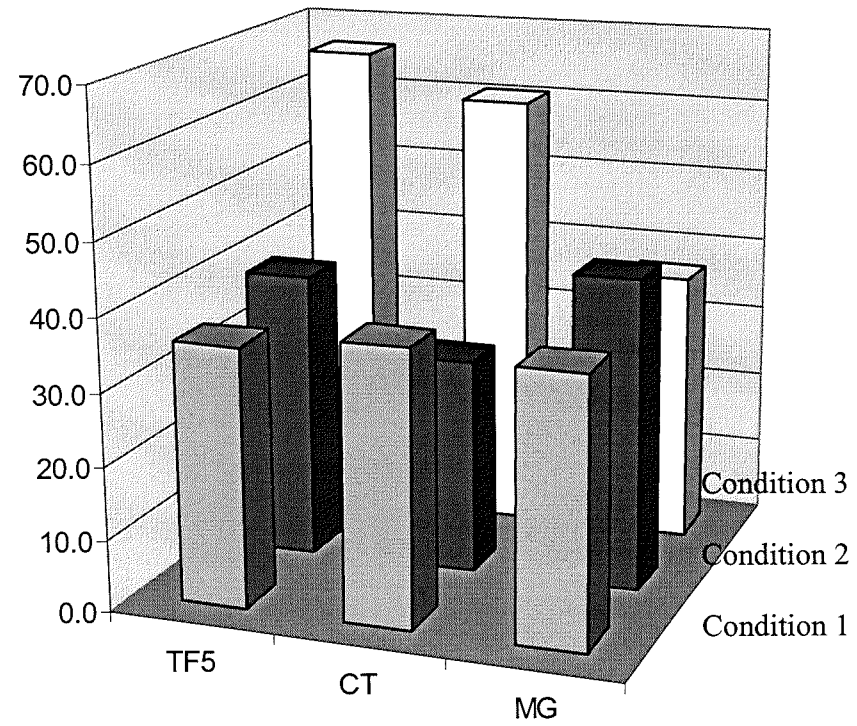


Figure 3-5. Mean CVs (%) for all measurements for all three instruments in conditions 1 – 3

Table 3-5. Intraclass correlations coefficients for reliability of each instrument in each condition.

Instrument	TF5			CT			MG			
	Condition	C1	C2	C3	C1	C2	C3	C1	C2	C3
LF		.95	.77	.87	.84	.90	.83	.85	.94	.86
LF(nu)		.51	.65	.16	.52	.82	.70	.80	.50	.52
HF		.79	.87	.65	.92	.92	.82	.71	.79	.47
HF(nu)		.74	.65	.73	.90	.82	.67	.86	.78	.56
LF:HF		.77	.71	.64	.89	.88	.82	.85	.82	.81
RMSSD		.88	.85	.69	.90	.91	.91	.93	.86	.79
SDNN		.57	.85	.83	.87	.67	.72	.77	.72	.76
Mean RR		.93	.89	.84	.99	.87	.86	.86	.93	.92

LF – low frequency spectral power, HF – high frequency spectral power, nu – normalised units, LF:HF – the ratio of low to high frequency spectral power, RMSSD – route mean square of the standard deviation of normal to normal interval differences, SDNN – the standard deviation of normal to normal intervals, mean RR – mean time (ms) between normal r-waves.

* Indicates result is significantly ($P < 0.05$) different from both other instruments.

In condition 3 (breathing) all frequency domain measures again, showed excellent levels of agreement. The SDNN measure from the CT was again shown to significantly exceed the value of this measure from the other instruments. Also in this condition, there was also a small, but statistically significant difference in the mean RR interval. *Post-hoc* analysis showed this interval increased systematically in all three instruments by a total of 5.6 ms from the CT (lowest) to the MG (highest).

3.3.2. *Reliability.*

The CV for each parameter measured in each position is displayed by instrument in Figures 2-4. From figures 2-4 it can be seen that the CVs for the eight parameters measured vary greatly from one another in terms of their reliability both within and between conditions 1-3. Within each condition it can generally be seen that CVs for time domain variables and frequency domain variables expressed in normalised units tend typically to be lower.

What can also be seen from the figures is that although the general pattern of reliability is similar between parameters and positions by instrument there are a number of very large CVs generated singularly by the TF5 and CT that differ greatly in comparison to parallel measures from the remaining two systems. For instance CV for the LF(nu) in the CT (235%) is much greater than the CV for LF(nu) recorded by the TF5 and MG (<20%).

In order to facilitate an overall comparison of the reliability of HRV measurements made by the three instruments the mean CV in each position and the overall mean CV are shown figure 3-5. From this it can be seen that the MG instrument demonstrated the lowest overall CV: the highest CV and therefore least reliable instrument was the CT. Between conditions 1-3, CVs obtained in condition 1 (Figure 3-5) were generally lower for each HRV measure than when subsequently measured in conditions 2 and 3. These lower CVs demonstrate greater reliability of the measurement of these HRV measures in the supine position.

Intraclass correlation coefficients were also calculated for the purpose of comparison of the present results with previous data expressed using this statistic. In this case the ICC for the repeated measurements made by each instrument under each condition were calculated. These data (Table 3-5) show the variable nature of the ICC coefficients. The ICC values range from excellent ($R = 0.99$) to very poor ($R = 0.16$). Although a poor R-value in one instrument is often accompanied by others from those remaining, this is not a uniform scenario.

3.4. Discussion.

3.4.1. Agreement.

The ICCs generated from the parallel acquisition of ECG data recording from the study participants clearly show excellent levels of agreement between the TF5, CT and CP instruments. In total, 24 ICCs were generated for eight HRV parameters measured under three different conditions. In all, twenty-three of these ICCs had lower bound 95% C.I. which were greater than $R = 0.75$. This has previously been deemed to represent agreement between methods in the measurement of the same quantity.

Another criterion sometimes used for supporting interchangeability is the similarity of the mean measured results obtained by the two methods (Lee *et al.* 1989). The results of the repeated-measures ANOVA and *post-hoc* paired *t*-test used to assess this highlighted several instances where parameters demonstrated significant differences. Specifically, mean NN interval and SDNN showed systematic differences between all three instruments during condition three. In condition two there were also differences between NN interval means (not significant) and again SDNN was found to differ significantly. In this instance, only one instrument (CT) was found to deviate from the other two.

The results of the ICC tend to suggest that all three instruments were comparable in their ability to record, process and interpret ECG data in terms of the variability of the NN intervals. The similarity of results suggest that the treatment of the data following recording, the removal of abnormal beats and where appropriate the interpolation of RR intervals (and creation of NN interval data) does not have a significant effect on the outcome of the HRV parameters measured. Additionally, it seems that the *post-hoc* processing of these data and the mathematical transformation of NN interval data into time and frequency domain HRV data is also very similar. The majority of data confirm that these three commercially available analysers show excellent agreement in the measurement of simultaneous HRV readings.

In terms of interpretation of these results it is difficult to make comparisons with previous data due to the lack of similarity between data collection protocols. The majority of literature concerning agreement of HRV instrumentation concentrates on the use of 24-hour ambulatory ECG recordings made on to Holter tape machines, and the subsequent analysis of these recordings via various HRV analysis instruments.

The data from the present study are in agreement with those of Simula *et al.* (1997). The conclusions of this study were that when analysed by the correct corresponding system, the 24-hour ECG recordings (analogue and digital) were highly comparable. The problems elucidated by Simula *et al.* (1997) when recordings from one system were analysed by another system were not assessed in the present study.

The current data also agree somewhat with those of Yi *et al.* (2000) who demonstrated high levels of agreement between a number of commercially available HRV analysis instruments using a series of pre-recorded Holter tapes. Although the agreement was not as good as demonstrated in the present study, the tapes analysed by Yi *et al.* were exposed to an extra process whereby variation prior to analysis could have occurred. Although trained technicians were used to annotate the tapes

and aid the automated protocols of the instruments in the removal of abnormal RR intervals, it was found that up to 75% of the variation between readings could be accounted for by interobserver differences.

These findings are also in agreement with those of a previous study (Kroll *et al.* 1996) in which it was demonstrated that a number of HRV analysis systems showed good levels of agreement. Kroll *et al.* (1996) specifically assessed the effect of interobserver differences on agreement between Holter tape analyses. It was determined that those time domain measures representative of the high frequency oscillations of RR intervals were particularly prone to disagreement. This was found to be due to the removal of differing numbers of shorter RR intervals during marked sinus arrhythmia as these cycles are attributed to ventricular ectopic beats by some but not all observers.

Unlike the data in the present study, Jung *et al.* (1996) demonstrated poor agreement between HRV parameters obtained from a single Holter recording. Again, the reason put forward for this by the researchers was the inconsistency in the removal of ectopic beats and artefacts from the RR interval data. In this case the researchers commented that the problem was not in the interobserver differences in beat removal. Instead a technical issue was raised. It was claimed that the systems used did not allow the necessary visual inspection of the RR interval data prior to processing and subsequent HRV analysis. Under such circumstances the user of the instrument has to rely solely on the automated software algorithms provided by the manufacturers to remove and interpolate RR intervals deemed abnormal. This can create variation in the raw ECG signal prior to any subsequent analysis.

A similar problem was encountered in the present study. Both the TF5 and the CP system allow visual inspection of the raw ECG signal. The systems also represent each RR interval measured either graphically (TF5) or in numerical form (CP). The algorithm for beat removal in the TF5 is fixed, whereas in the CP it is variable. By default, both these instruments interpolate and replace abnormal RR intervals.

Although the CT allows observation of the tachogram for visual inspection, it does not facilitate the *post-hoc* removal of ectopic beats by the researcher. Instead it simply reports the total number of beats sampled and the number rejected.

In order to ensure that each system was assessed as equally as possible it was decided that no *post-hoc* manual removal of beats would be undertaken in the present study. Non-parametric analysis (Kruskal-Wallis) demonstrated that a small but significantly greater number of beats was removed by the CP (0.89) compared to the TF5 (0.5) during the 5-min of ECG data collection under condition 3. The CT however was found to have removed a much greater number (7) of beats compared with the other two instruments during this time.

Further analysis of the tachogram revealed that there were in fact significant differences in the actual number of RR intervals being sampled by each instrument under certain conditions.

In the condition 3 there were systematic difference in the mean RR interval reported by all three instruments. This finding was not wholly unexpected. It was initially assumed that the raw RR interval data obtained from the TF5 would differ from the other two instruments because the collection interval for the TF5 is always the longer option from 300 s or 300 beats. When the mean RR interval of a subject is greater than 1000 ms the TF5 will sample more beats than the CT and CP instruments. The data collection window of the latter two instruments is set at 300 s.

An unexpected finding was that all the instruments differed significantly from each other in the number of RR intervals sampled. More surprisingly the biggest difference was between the CT (414.3) and the other two remaining systems (TF5, 335.8; CP, 314.8). Further visual analysis of the tachogram from the CT showed a discrepancy between the assumed time window of ECG recording and that which actually occurred.

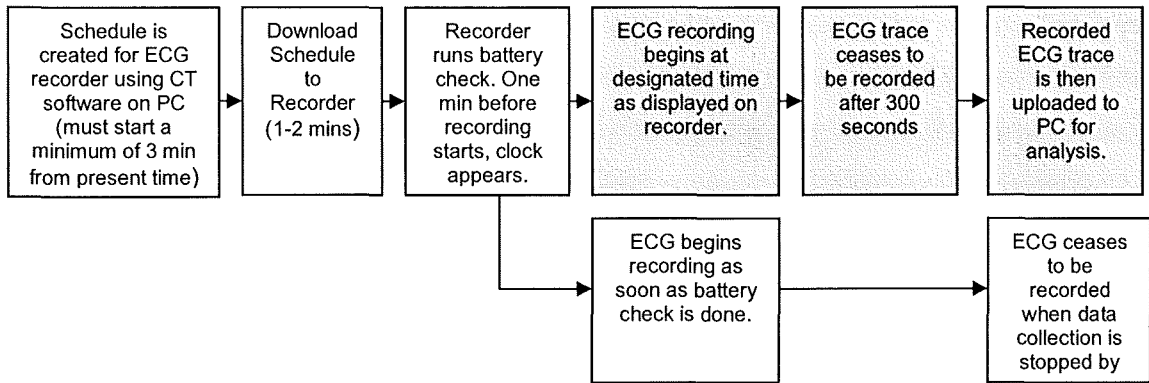


Figure 3-6. Supposed (top route) and actual (bottom route) process of data collection by CT instrument.

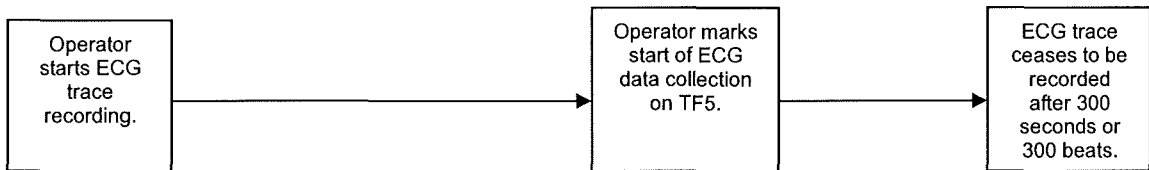


Figure 3-7. Actual process of data collection by TF5 instrument.

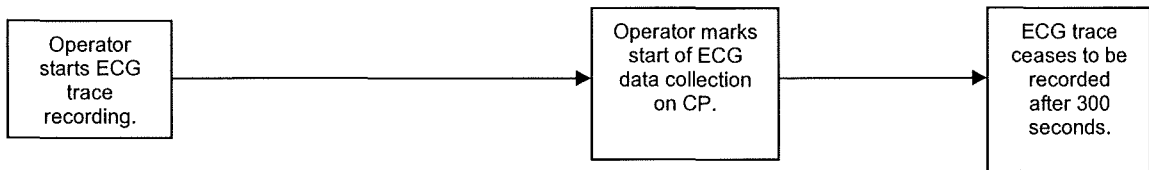


Figure 3-8. Actual process of data collection by CP instrument.

Figures 3-6 - 3-8 show the processes by which the exact timing, for recording of the ECG signal is obtained. The CT was used at the marker for the start point of the other two ECG recordings as it was subject to a ‘countdown’ and the other two were manual in nature. From the observed discrepancies in the assumed and actual data collection protocols it can be seen that the TF5 and CP instruments still recorded the same ECG data, except where the TF5 collected data over a longer duration due to the mean RR interval exceeding 1000 ms.

Despite the above discrepancies, the majority of frequency domain parameters demonstrated excellent agreement. This it to be expected despite the differences in raw RR signal recorded by each instrument. The RR intervals may have been drawn from slightly different segments of the same ECG but as they represent sampling

from a stable ECG they should demonstrate similar patterns of frequency oscillation. The similarity in HRV parameters derived from several short-term segments of a single ECG has been shown previously (Ponikowski *et al.* 1996; Marks and Lightfoot 1999). In the present study this demonstrates the stability of the tachogram, which is a prerequisite for HRV analysis. Alternately, it helps to demonstrate where some of the small variation found between the instrumentation may have come from.

Unacceptable agreement (lower 95% CI, $R < 0.75$) was demonstrated in only one variable (LF:HF ratio) and only in condition 2. To explain this, the nature of the variable itself must be examined. The LF:HF ratio is dependent on the raw units scores of low frequency (LF) and high frequency (HF) oscillations. Small variations in either of these values affect the ratio, which is therefore prone to summative

variation from both sources. The fact that the lowest agreement was found in the standing position reflects the dynamic variation which occurs in this specific variable. It is important to note that the CT instrument deviates significantly from the remaining two instruments and differences in sampling epoch as described earlier may account for variation in the LF:HF ratio. Specifically the CT was found to begin sampling earlier than the other systems. In this condition this would mean it sampled ECGs during a period where the tachogram was still stabilizing. Visual analysis of the raw tachogram signal confirmed this suspicion.

Figure 3-9 is derived from the initial model of HRV data collection published by Kennedy (1996). It gives a visual representation how the three systems used in the present study. The shaded areas represent differences in the HRV data collection and interpretation process. The italicised boxes represent data at various stages of processing. These demonstrate where the differences in processing by the systems are translated into differences in data.

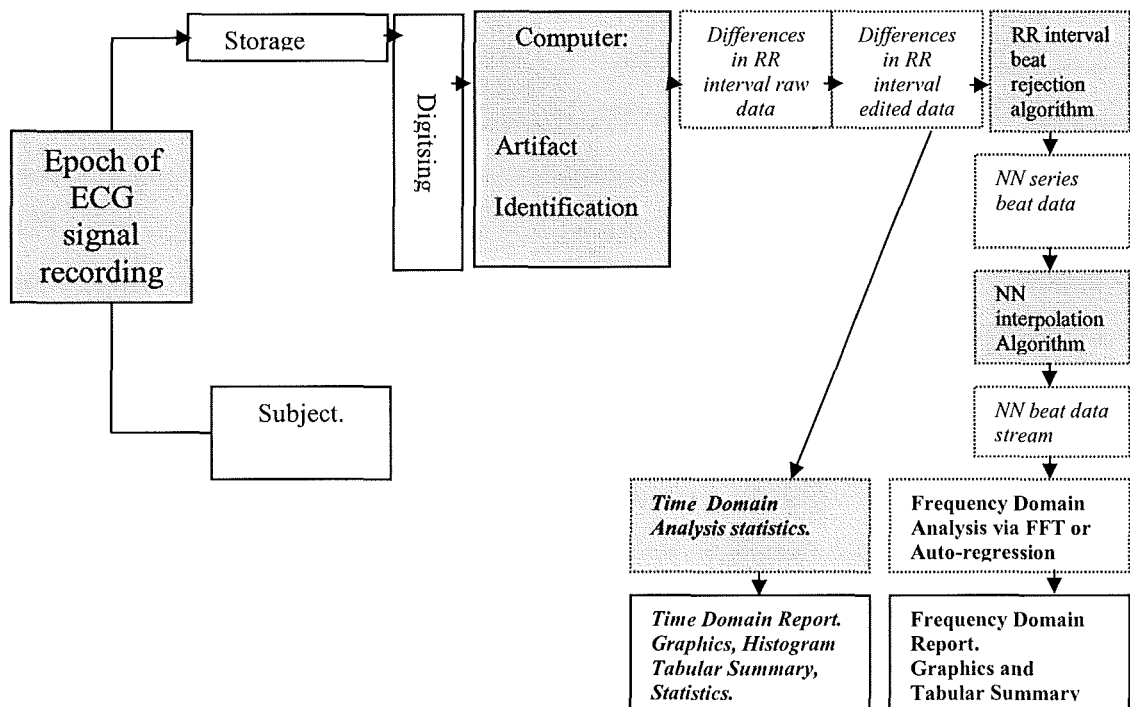


Figure 3-9 Identification of differences in data collection and analysis.

This model demonstrates how frequency domain measures remained very similar between the instruments despite differences in the raw RR and later NN data entering the analysis.

3.4.2. *Reliability.*

Overall CVs for HRV parameters in the present study showed a wide variation, ranging from <1% to >100%. The initial aim of this study was to assess the reliability of each data collection instrument. The large range of values obtained made this very difficult. Closer observation of figures 3-1 - 3-3 shows that for the majority of HRV measures made, the CVs generated showed similar fluctuations. For example, large CVs were recorded by all instruments for LF:HF ratio in the standing condition and small CVs were reported for RR interval in the supine condition. There are also deviations from this pattern where data from one instrument created a large CV which did not agree with the remaining two. An example of this is the CV generated by the CT for LFnu in the supine condition. These discrepancies can be partially explained again by the differences in actual ECG sampling epochs in the CT system. This can also explain a number of the large CVs found using this instrument.

3.4.2.1. *Reasons varied CV values.*

To explain variation from test to retest using the other two systems one has to look at the similarities between CVs generated by the TF5 and the CP. The fact that the size of the CVs tend to follow each other from parameter to parameter and from condition to condition suggest that it is not the systems *per se* which are responsible for test-retest differences but biological variation in the subjects. In order to assess the CVs generated in the present study comparison with previous investigations using similar methodologies is necessary. Using short (10-min) sequential ECG recordings from patients lying supine and anaesthetised it would be expected that CV would be low. Freed *et al.* (1994) demonstrated CV from 5-16% and these are indeed lower than the range of CVs from the

comparable (supine) condition in the present study (TF5 = 5.5-125%; CP = 8.5-94.7%). The usefulness of determining the reliability of HRV from sequential ECG recordings (Freed *et al.* 1994) is questionable except in the determination of a baseline for test and maneuver HRV analysis.

3.4.3. *Comparisons with previous data.*

Using an identical data collection protocol to the supine condition in the present study, CVs from 25-139% for time domain and 45-111% for frequency domain measures have previously been reported (Ponikowski *et al.* 1996). These are, overall, slightly higher than those generated by all three systems analysed in the present study. The authors suggested that good reliability of HRV measures should not be taken for granted in research and suggested that research groups should report reliability coefficients as part of justification of sample sizes used.

Lord *et al.* (2001) studied the reliability of the LF oscillations in the frequency domain to assess this variable's usefulness in intervention studies. The LF component was found to show poor reliability in both normal subjects (CV = 45%) and in heart transplant recipients (CV = 76%). By making repeated measures at three specific times, a significant effect due to time of day was demonstrated. Time of day and a training effect were presented as underlying factors in the high CVs found. The CVs for this particular variable under similar data collection conditions in the present study were 170.8% (TF5), 52.5% (CT) and 18.3% (CP). These coefficients are widely spread and not comparable to those of Lord *et al.* except that they are (excluding, CP) undesirably high. Lord *et al.* (2001) warned that longitudinal changes in HRV due to intervention should be interpreted with caution. Data from the current study support this notion although it should be noted that the recommendations of Lord *et al.* are based on the reliability of measures from denervated hearts after transplantation and should not, therefore, be generalised. As discussed previously (Ponikowski *et al.* 1996) the effect of poor levels of reliability on necessary sample size for

interventions studies was also raised by Lord *et al.* and the data presented herein confirm that this may be problematic.

The findings of the present study show generally higher CVs than those reported in a similar study (Sinnreich *et al.* 1998). In this study, CVs from data recorded in the supine position ranged from 6% (SDNN) to 12.1% (HF) and were similar during controlled breathing (6.1%, SDNN - 10.7%, HF). These coefficients are lower than those of any instrument in the present study and may be explained by certain methodological differences. Sinnreich *et al.* (1998) used a Marquette 8500 Holter system to make the ECG recordings, a system previously demonstrated to have moderate reliability during 24-hour recordings (Kautzner *et al.* 1995). Processing of the RR intervals included automatic and manual editing of the ECG by a skilled operator whereas the present study deliberately relied only on automatic processing.

The spectral decomposition of the NN data by Sinnreich *et al.* was carried out using autoregressive modeling as opposed to fast Fourier transformation (FFT) in the present study. This method determines spectral amplitude peaks as well as power spectral densities for each data set. This eliminates possible variation from peaks crossing boundaries between LF and HF due to variation in breathing frequencies. In the present study this phenomenon was noted in a number of subjects in the supine position. Sinnreich *et al.* employed a familiarisation protocol with each subject. This was not true of the present study. Finally, both data collection conditions in the study by Sinnreich *et al.* involved the subject lying supine. It may be that the slightly lower CVs in the controlled breathing condition reported by Sinnreich *et al.* are due to increased subject relaxation increased protocol familiarity. This may be particularly true of the controlled breathing data collection condition, which always followed the spontaneous breathing condition.

In the present study the lowest overall CVs were found in the first data collection condition, supine. Data collected in both the standing and controlled breathing

conditions showed poorer overall reliability. In these conditions the subject had a shorter familiarisation or adjustment time and in both cases had recently moved either from a supine to a standing position or vice versa.

From the discussion of the data from the present study, especially in light of the findings of Sinnreich and coworkers several recommendations can be made regarding data collection methodologies in the analysis of HRV.

The poorer reliability demonstrated in the standing condition and in the controlled breathing may be addressed in two ways:

1. Unless data are specifically required in the standing position data collection should be restricted to one position, lying supine with or without controlled breathing.
2. If a standing measure is deemed necessary a longer adjustment period than that in the present study (3-min) between changes in posture should be used.

In the case of all HRV measures a familiarisation period should be employed with all study participants, particularly for data collection during controlled breathing to which the subjects may not be accustomed.

Marks *et al.* (1999) used a highly controlled methodology to assess HRV reliability. They calculated HRV data on a 5-min data section taken from a total of 20-min of resting ECG. Controlled breathing (12 breaths·min) was used throughout the data collection and the small number of subjects used, (n=8) were all highly physically active, female, non-smokers. Each subject was checked for exposure to cigarette smoke using a carbon monoxide meter. Interestingly, one of the reasons cited for performing this study was to assess the practicality of using HRV as a regular screening tool in a clinical setting. In such a setting the degree of control exerted over the recording protocol in this study would be impractical.

3.4.4. *Recommendations.*

Overall it is recommended that studies of the reliability of HRV data should be specific in their aims. If the reliability coefficient gained is to be used to assess the effectiveness of a specific intervention in a controlled scientific investigation then the most reliable estimates of HRV possible should be pursued through the use of scientific control and rigour. If HRV is to be used as a general screening tool on a daily basis in an office or surgery, a pragmatic approach to reliability will result in the production of a more practicable level.

As part of a laboratory study subjects should be tested at a similar time of day on all visits. They should have fasted overnight and should have had no tobacco or caffeine containing substances on the day of the test. Alcohol consumption should also be avoided on the day of the test and certainly limited on the night prior to testing. There is some evidence that, in female subjects, phase of the menstrual cycle should also be accounted for (Yildirim *et al.* 2002), but this may not be feasible to control for in a clinical setting. All of the above parameters should be recorded along with the HRV data as they may help to account for anomalous results.

Ponikowski *et al.* (1996) correctly noted that good reliability is essential for maximal utility in clinical research. There is an inverse relationship between the statistical power of a study and the square of the reliability coefficient for a given sample size. Conversely, for a given statistical power, the necessary sample size increases in exponentially to the size of the reliability coefficient.

3.5. **Conclusions.**

3.5.1. *Agreement.*

Agreement between all three instruments was excellent. Where there were significant differences between the measures (RR interval, SDNN) this was due

to differences in the raw data sampled. From these findings it can be concluded that the instruments studied may be used interchangeably with one another. Each of the instruments used in the present study has advantages and disadvantages.

The highest degree of control over data collection is afforded by the CP system. This system allows automatic and manual data editing. It also records and displays the full ECG signal to allow full visual inspection. The main problem associated with this system is that it is static in nature and only suitable for laboratory based investigation. It is also the most expensive of the three instruments used.

The TF5 system also facilitates the visual inspection of the ECG signal. This system also allows automatic and manual editing of the data. This system has a major advantage of being portable is therefore ideal for use in clinical as well as laboratory settings. The TF5 is the middle-priced of the three systems.

The CT system is also portable. This ambulatory data collection instrument is specifically designed for 24-hour data collection protocols. It can be used for shorter collection times but because of its intended use it does not store full ECG traces, and does not permit manual editing of the data. This instrument is, however, considerably less expensive than the CP and TF5 systems.

3.5.2. *Reliability.*

The large CVs found in the present study and the similarity in patterns between HRV measures between conditions, and instruments suggests that biological variation was the major source of variation between test and retest.

From the existing literature and the data from the present study it is suggested that the calculation of study-specific reliability coefficients (ICC, LoA, CV) be undertaken. This should be done using data collection conditions similar to those to be employed in the study. A similar sample to the study population should be

used and this should be done for each HRV parameter to be included in the study. On the basis of these coefficients, researchers should then calculate necessary sample sizes or at least give estimates of statistical power in each published study.

Appendix 3 contains a number of sample size calculations for possible future studies using HRV parameters used in accordance with the Taskforce (Camm *et al.* 1996) recommendations. The HRV parameter values are taken from the published literature and the sample sizes have been modified by the CVs obtained in the present study (TF5, CT or CP).

3.6. References.

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CHAPTER 4. HEART RATE VARIABILITY AND EXERCISE.

Abstract.

The aim of this chapter was to systematically review the literature concerning measures of heart rate variability (HRV) and their relation to acute and chronic exercise.

Using cross sectional comparisons, it can be shown that measures of HRV, particularly those associated with vagal modulation are commonly found to be significantly greater in elite athletes than in sedentary controls. When comparisons are made between individuals in groups based on levels of activity or fitness who are not elite athletes, this difference is obvious. Longitudinal studies, which have used exercise as an intervention to alter HRV show highly heterogeneous results. Interpretation of the findings of such studies is made difficult by the many methodological disparities.

When measured during exercise, there is a large and statistically significant drop in global measures of HRV. These measures tend to decrease linearly as a function of exercise intensity. Numerous studies have shown that measures found to adequately describe vagal and sympathetic ANS modulation at rest, perform poorly under exercise conditions. This is particularly true at higher exercise intensities.

In conclusion, studies into the longitudinal effects of exercise interventions on resting measures of HRV give heterogeneous findings and more research is needed in this area to clarify this issue. During exercise, recommended HRV measures, commonly used at rest, do not adequately describe the ANS response to exercise. Further work to evaluate these measures and possible alternatives is, therefore, required.

4.1. Introduction.

During exercise it is known that heart rate (HR) increases linearly with work rate and that the chronotropic response of the heart during exercise is influenced by the subject's training state. At rest, it is known that endurance trained individuals display a distinct, resting bradycardia compared with untrained controls (Ekblom *et al.* 1973; Lewis *et al.* 1980)

The interaction of the sympathetic (SNS) and parasympathetic or vagal (PNS) branches of the autonomic nervous system and their control over HR has been of interest to researchers for over 100 years. Historically, surgical procedures using animal models (Rosenblueth *et al.* 1934) and pharmacological treatments of both animals (Hughson *et al.* 1977; Ordway *et al.* 1982) and humans (Sutton *et al.* 1967; Lewis *et al.* 1980; Katona *et al.* 1982) have been employed to quantify the influence of the SNS and PNS on HR at rest and in response to acute and chronic exercise.

Surgical and pharmacological procedures are both invasive and expensive, making a non-invasive method of studying autonomic control desirable. To study PNS control over HR, the use of the alterations in RR interval during respiration (al-Ani *et al.* 1996) was proposed and developed (Katona and Jih 1975; Fouad *et al.* 1984). The alterations in RR interval during respiration were termed the 'variation in heart period' or 'VHP' (Kenney 1985) and although sharing many common features should be thought of as distinct from 'heart rate variability' as it will be discussed here.

Heart rate variability (HRV) is defined as: "The cyclical changes in sinus rate over time." (Ori *et al.* 1992). It is a non-invasive tool which can be used to study the autonomic control of HR (Akselrod *et al.* 1981; Pagani *et al.* 1986; Malliani *et al.* 1991; Dreifus 1993; Camm *et al.* 1996). For a technical review of the use of HRV the reader is directed to the report from the Task Force of the European Society of Cardiology and the North American Society of Pacing and

Electrophysiology (Camm *et al.* 1996) although several other excellent reviews spanning over 30 years of research are available (Luczak and Laurig 1973; Sayers 1973; Ori *et al.* 1992; Stein and Kleiger 1999; Pumprla *et al.* 2002; Routledge *et al.* 2002)

The aim of the present review is not to outline the many applications of HRV, but to concentrate on its use in providing insight into the acute and chronic effects of exercise on autonomic control of the heart. The validity of HRV to represent autonomic balance fully has been discussed in detail elsewhere (Pomeranz *et al.* 1985; Pagani *et al.* 1986; Malliani *et al.* 1991; Montano *et al.* 1994; Camm *et al.* 1996; Pagani *et al.* 1997; Warren *et al.* 1997; Goldberger 1999; Houle and Billman 1999; Stein and Kleiger 1999) and discussion of this, and technical procedures will only be undertaken when pertinent. A full list of abbreviations and technical terminology can be found in appendix 1 and where technical terms are used for the first time in the text they are defined.

4.2. Assessment of autonomic function in athletes and controls at rest using HRV.

Surgical and pharmacological studies of autonomic regulation lead to the classical view that the resting bradycardia observed in trained individuals was due to increased PNS activity. This view was supported by empirical research in humans (Frick *et al.* 1967; Ekblom *et al.* 1973; Smith *et al.* 1989) and in a theoretical review (Scheuer and Tipton 1977). Evidence refuting this claim was also provided empirically (Sutton *et al.* 1967; Lewis *et al.* 1980) and this opinion also has an accompanying review (Badeer 1975). Katona *et al.* (1982) stated that several authors (Raab *et al.* 1960; Frick *et al.* 1967; Ekblom *et al.* 1973) may have misinterpreted pharmacologically derived data. A paper frequently cited as supporting a parasympathetic influence on resting bradycardia is that of Ekblom *et al.* (1973). Closer scrutiny of this paper reveals that although the authors make postulations supporting this notion, they are not supported by data. Data gained during atropine infusion showed some evidence for increased PNS activity after

training in only five out of seven subjects. During exercise no effect was evident. Evidence from propranol infusion clearly showed decreased SNS activity at rest and during exercise. From the evidence using double blockade the authors concluded that decreased PNS drive was responsible for the observed bradycardia. The authors do state:

“The well known decreased HR with increased physical fitness to be due to reduced beta-adrenergic receptor activity in combination with increased parasympathetic activity.”

They do not however, support this statement with any of their own data. It is therefore possible that subsequent authors have misinterpreted this statement as empirical support for a parasympathetic origin of bradycardia due to training.

A number of alternative explanations for the commonly observed bradycardia in athletes exist. These include alterations in the mechanical strain put on the pacemaker cells by cardiac hypertrophy (Lewis *et al.* 1980) metabolic alterations in the pacemaker cells and neurotransmitter sensitivity (Ekblom *et al.* 1973; Katona *et al.* 1982). It has also been suggested that genetic factors may be important (Smith *et al.* 1989).

It is clear from the data presented that the study of autonomic control of HR by surgical and pharmacological methods has failed to identify a single cause of bradycardia. What follows is a review of how non-invasive measures of VHP and HRV have made additions to knowledge concerning resting bradycardia in relation to chronic exercise levels.

Kenney (1985) demonstrated a significant correlation ($r = 0.92$, $P < 0.001$) between $\dot{V}_{O2_{max}}$ and VHP in 21 healthy subjects. By dividing subjects into high, moderate, and low fitness groups based on $\dot{V}_{O2_{max}}$, significant differences between amplitudes of sinus arrhythmia were demonstrated. It was concluded that age related decrease in sinus arrhythmia may be due to concomitant decreases in activity and not due to ageing *per se* as previously claimed

(Hrushesky *et al.* 1984). This finding was later supported by longitudinal data from the same laboratory (Schraufek *et al.* 1990).

Maciel *et al.* (1985) compared respiratory sinus arrhythmia (RSA) between athletes and sedentary controls. To test the theory further that training-induced increases in PNS activity were responsible for resting bradycardia in athletes, pharmacological parasympathetic blockade was achieved via atropine infusion. No significant difference was found in RSA amplitude between groups. No comparison of Δ HR due to atropine infusion between groups was made. In athletes, RSA amplitude was found to correlate negatively ($r = -0.69$) with $\dot{V}O_{2\max}$. The statistical nonsignificance of this relationship was probably due to the small ($n=7$) sample size. The authors concluded that their data indicated no increase in resting activity of the PNS due to exercise. These findings should be treated with caution due to the small sample sizes.

Reiling and Seals (1988) found significantly longer RR intervals but similar levels of RSA in athletes and controls as measured by maximum to minimum RR interval differences. Standard deviation of the RR intervals (SDRR), which is a time domain measure of global HRV was also similar (72 ± 15 vs. 70 ± 9 ms) in the two groups. The results may be due to RSA possessing insufficient sensitivity to detect differences in such groups. Certainly, SDRR gives only a global indication of HRV and does not differentiate between PNS and SNS influence of HR. A more detailed analysis of autonomic function may have revealed differences between groups.

In contrast to these findings, Dixon *et al.* (1992) compared highly trained runners (>50 miles per week) with sedentary controls using autoregressive power spectral analysis of short-term resting ECG trace. It was found that the athletes had significantly greater high frequency spectral power (HF), reduced low frequency spectral power (LF) and a lower LF:HF ratio compared with controls while lying supine. HF, LF and LF:HR were used as markers of PNS activity, SNS activity

and autonomic balance respectively. It was concluded that the greater HF component in athletes gave evidence of enhanced vagal tone and that training modifies HR control in part, through neurocardiac mechanisms.

Gallagher *et al.* (1992) found that LF, HF and LF:HF derived from autoregressive spectral analysis revealed significant differences in level and balance of autonomic control between groups. A significantly elevated RSA in trained vs. untrained individuals was also found, demonstrating a shift towards what was termed a 'parasympathetic cardioprotective balance'. These findings are at odds with the HR data reported, which show the trained group to have a higher resting HR (71 bpm) compared with the two remaining groups (69 and 54 bpm). The authors erroneously stated that this demonstrated resting bradycardia in their subjects.

The studies reported so far have all used short-term data collection methods. Data on HRV can also be obtained by ECG monitoring over 24-hours. Goldsmith *et al.* (1992) utilised 24-hour monitoring to study autonomic activity in endurance-trained men and sedentary controls. They found that the pronounced bradycardia shown in trained individuals was accompanied by greater SDNN, total spectral power (TP)*, LF and HF. Additionally, HF made up a larger percentage of TP in trained (3%) vs. untrained (1.3%) subjects. This was interpreted as demonstrating predominating vagal activity. The increased LF was interpreted similarly, although the reason given for this interpretation was somewhat circular. The authors seem to develop their hypotheses on their own findings stating that if LF power was sympathetic in nature then it would be higher in untrained subjects. As LF was found to be lower in trained subjects they concluded it to be vagal in nature.

There is considerable debate over the origin of LF fluctuations in RR interval. Some authors (Pomeranz *et al.* 1985; Rimoldi *et al.* 1990; Malliani *et al.* 1991; Kamath and Fallen 1993; Montano *et al.* 1994) support the view that it is purely a measure of SNS activity whereas others (Akselrod *et al.* 1981; Appel *et al.*

1989; Stein and Kleiger 1999) believe it to be influenced by both branches of the ANS. Camm *et al.* (1996) have also reinterpreted evidence from authors of the former viewpoint (Pomeranz *et al.* 1985; Malliani *et al.* 1991) which are reported as supporting a combined PNS and SNS contribution to LF.

Janssen *et al.* (1993) compared selected spectral and time series measures of HRV in highly trained cyclists and sedentary controls. Fast Fourier transformation (FFT)* was used to determine LF and HF spectral power; LF was also calculated in normalised units (nu)*. In the supine position the cyclists showed significantly longer RR intervals, a reduced LF (nu) and greater pNN50% than controls. No differences were found between coefficient of variation (CV)* or standard deviation SD* of the RR interval data. Both groups showed significant alterations in all measures (except CV) during a transfer from the supine to the standing position. The magnitude of this change was greater for RR interval, LF (nu) and pNN50% in athletes. The supine data were interpreted to indicating an increased level of PNS (or decreased level of SNS) activity in athletes. The differences in response to standing were said to be indicative of better quality autonomic control in the athletes.

The authors state that LF and HF were calculated; this is necessary to calculate LF(nu) used here as recommended (Malliani *et al.* 1991) as a measure of purely sympathetic activity. Interestingly, HF, which is commonly used as an indicator of PNS activity, was not reported in raw or normalised units. Instead, the time domain measure of pNN50% was reported. This makes an unusual mixture of time and frequency domain measures. Although pNN50% is thought to give some inference of PNS activity it is currently recommended that when using short duration recordings, frequency domain measures should be used in favour of time domain alternatives (Camm *et al.* 1996). The authors give no justification for this choice of measures.

Puig *et al.* (1993) reported significantly greater variability in all time domain measures when comparing athletes with controls when measured in the supine position (15 min duration). Using FFT they also reported increased TP, LF and

HF spectral components (raw units) in athletes. Unlike Janssen *et al.* (1993) LF (nu) was found to be similar in athletes (20.4) and controls (20.7). It was concluded that these results demonstrated an increase in parasympathetic activity without reduction in sympathetic tone.

In paradox to the above findings, DeMeersman (1993) analysed the RSA amplitude of active individuals and matched controls (n = 146) divided into six age categories from 15-75 years. In both groups RSA declined with age but that in each age category active subjects displayed significantly greater amplitude of RSA. It was concluded that exercise plays an important role in augmenting and maintaining HRV. In agreement with these findings, Sacknoff *et al.* (1994) demonstrated increases in the time domain measures of SDRR and pNN50 in athletes compared with control subjects. This is in contrast with frequency domain analysis of the same data which paradoxically showed attenuated levels of TP, LF and HF in the athletes compared with controls. The authors noted that the correlations typically observed between time and frequency domain measures (See Appendix 1) were not present in athletes. It seems more likely that what was interpreted as low levels of HRV in athletes may be an artefact of the extremely large values for TP, LF and HF reported for the control groups. Unfortunately, no physical description of either group is given (e.g. bodyweight, activity level, $\dot{V}_{O_2 \max}$), as this may have helped to explain the spectral values obtained from the 15-min ECGs analysed which are up to ten times larger than normal values available in the literature (Camm *et al.* 1996).

Comparing physically active postmenopausal women with sedentary aged matched controls it was found that all time and frequency domain measures (with the exception of LF:HF ratio) of HRV were augmented in active women (Davy *et al.* 1996). Augmented parasympathetic activity and reduced sympathetic outflow were evident in the trained group. Spontaneous cardiac baroreflex sensitivity (SBRS) was found to account for the majority of the variability in HF power.

Macor *et al.* (1996) found no statistically significant differences in spectral components (raw units) between highly trained cyclist and controls. Analysis of HF (nu) revealed a significantly greater contribution to autonomic balance by the PNS in cyclists compared with controls. The authors concluded that competitive cycling caused an increase in vagal traffic to the SA node. Returning to the raw units for spectral powers reported, large differences were clearly evident in LF (Controls: 381, range 15-9809 ms² vs. cyclists: 933, range 116-7525 ms²) and HF (Controls: 730, range 10-8318 ms² vs. cyclists: 890, range 87-3981 ms²). The lack of statistical significance in these measures may be an artefact of the analysis used. Although the researchers do not state which measures of central tendency are reported. These appear to be given as mean values. Assuming this, the data display a strong positive skew and wide, heterogeneous dispersions of LF and HF..

Skewed distributions are commonly reported in the literature (Bigger *et al.* 1993; Bigger *et al.* 1995). This, and heterogeneous variances may preclude the use of parametric statistical analysis. The analysis of the data by Macor *et al.* (1996) using (parametric) independent *t*-tests may therefore, be inappropriate.

A similar problem is evident in a further study (Lazoglu *et al.* 1996), this time comparing cyclists, weight lifters and sedentary controls. No statistically significant differences were found in either time or frequency domain HRV measures derived from 24-hour Holter monitoring. There are three methodological problems with this study. Firstly, the cyclists are defined by $\dot{V}O_{2\max}$ (> 55 ml·kg⁻¹·min⁻¹), the weight lifters by duration of activity (> 5 h·week), the sedentary group are not defined. Secondly, ANOVA was used to identify differences between these groups despite the large intergroup differences in SDs reported. This heterogeneity of variances violates one of the assumptions underlying the use of ANOVA, greatly reducing the differential power of the statistic. Thirdly, the cyclists did not display the significant bradycardia that would be expected based on previous data when compared with either weight lifters or controls. This may indicate a degree of similarity in the three groups.

Although not reported, it was likely to be based on previous evidence that the distributions of spectral measures from 24-hour recordings were skewed, but no statistical assessment of the distributions was given in the methods section. These studies (Lazoglu *et al.* 1996; Macor *et al.* 1996) demonstrate the importance of correct data treatment and analysis when utilising such a sensitive measure as HRV. In light of such shortcomings inferences made on the basis of these studies should, at best, be cautious.

A further method by which the RR time series may be transformed into the frequency domain is coarse graining spectral analysis (CGSA) (Yamamoto and Hughson 1991). This method disregards all non-harmonic or fractal power in the frequency spectrum and analyses only the harmonic components. It provides different measures to those derived from methods of general spectral analysis (GSA). A full list and definition of these measures can be found in appendix II. Gregoire *et al.* (1996) used this method to identify differences in total power (TP), the parasympathetic nervous system indicator (PNS) and the sympathetic nervous system indicator (SNS) between four groups of trained and untrained individuals defined by age and sex. TP was almost three times greater in trained, mature females compared with untrained, mature controls. This difference was accompanied by a significantly (greater than four fold) reduction in SNS indicator value. Although these findings are similar to those reported previously (Macor *et al.* 1996) they are at odds with the majority of studies inasmuch as no differences between either TP, SNS indicator or SNS indicator were found between trained and untrained groups of: young males, mature males or young females. The lack of any statistically significant differences in the young group may be due in part to the relative fitness of the untrained group as indicated by their $\dot{V}O_{2\text{ peak}}$. Recently the sensitivity of spectral measures derived from CGSA has also been questioned (Myslivecek *et al.* 2002).

A second study using 24-hour Holter monitoring (Jensen-Urstad *et al.* 1997) compared highly trained runners with sedentary controls. It was found that

runners demonstrated greatly increased time and frequency domain measures of HRV over the whole period. When the period was divided into night and day periods, these differences persisted for all measures in the night period. This pattern was continued into the day period for all measures except HF which, although it was almost doubled in the runners, failed to reach statistical significance. The lack of significance may in part, be due to the small, unequal sample sizes in the groups (runners $n=16$, controls $n=13$). Using a similar methodology, Bonaduce *et al.* (1998) compared 15 male cyclists with age-matched controls. Although this cross-sectional comparison was made when the cyclists were in what was described as a 'detrained state' (1 month without vigorous training) they still displayed significantly greater values for all time and frequency domain measures of HRV than controls. Following logarithmic transformation of the data, all frequency domain measures were significantly increased in the cyclists. The magnitude of the difference in HF was actually less than that reported previously (Jensen-Urstad *et al.* 1997), illustrating the importance of statistical treatment of HRV data which commonly displays skewed distributions.

Analysis of HF (nu) and LF (nu) derived from autoregressive spectral analysis of 10-min sitting ECGs revealed a parasympathetic predominance in athletes compared with controls as indicated by increased HF (nu) (Shin *et al.* 1997). LF (nu) was also decreased in athletes compared with controls but this difference was not statistically significant ($P=0.07$). The authors did not supply data for LF and HF raw units or TP therefore inference regarding magnitude of HRV in athletes and controls is not possible.

In three groups of age-matched subjects categorised as poor, average and good $\dot{V}O_{2\max}$ Tulppo *et al.*(1998) found no significant difference in parasympathetic activity measured by the two dimensional vector analysis of Poincare plots* or HF CCV%*. Similarly, Melanson (2000) studied differences in HRV in three groups of subjects determined by their levels of self-reported physical activity.

Significant differences were found in the group reporting the lowest level of physical activity. This group had significantly lower HRV than the moderate and high exercise level groups for all time and (log transformed) frequency domain measures. The lack of a dose-response relationship was attributed partially to the fact that HF in particular may have been depressed in the high activity level group by previous exercise. The inability of HRV parameters to predict RR interval duration when regression analysis was applied to the data was interpreted as demonstrating a lack of relationship between parasympathetic activity and RR interval.

Migliaro *et al.* (2002) observed no statistically significant differences in HRV parameters between active and sedentary young adults. It should be noted that the exercise group appear to be only recreational athletes although no physiological data for either group was presented. It is therefore difficult to compare the findings of this study with the remainder of the literature, which typically reports the effect of training in athletes or groups clearly defined by physiological characteristics such as $\dot{V}O_{2\max}$.

In summary, the majority of studies show significantly greater PNS activity in trained vs. untrained individuals (Kenney 1985; Dixon *et al.* 1992; Gallagher *et al.* 1992; Goldsmith *et al.* 1992; De Meersman 1993; Janssen *et al.* 1993; Puig *et al.* 1993; Sacknoff *et al.* 1994; Macor *et al.* 1996; Davy *et al.* 1997; Jensen-Urstad *et al.* 1997; Shin *et al.* 1997; Melanson 2000). Although some studies report no significant differences in markers of PNS activity (Maciel *et al.* 1985; Reiling and Seals 1988; Tulppo *et al.* 1998; Migliaro *et al.* 2001), this can often be attributed to methodological and statistical shortcomings of the studies. Where high-level athletes who undertake high volumes of intense training are compared with controls, differences in autonomic function are evident. Where comparisons between 'active' and 'inactive' individuals are made differences are less obvious, even when the groups are clearly defined by an accepted physiological marker such as $\dot{V}O_{2\max}$.

Caution must be employed when making inferences from cross-sectional studies as confounding variables such as self-selection, and genetic influence cannot be controlled for. Although patterns in the data may emerge, it is impossible to infer causality from such a study design. For this reason a number of longitudinal studies have been carried out to assess pre and post-training alterations in ANS activity in a variety of populations.

4.3. Longitudinal data concerning the effects of exercise on HRV.

Elements of data from a number of studies cited in the following section have already been presented in section one as some studies have utilised combined, cross-sectional and longitudinal designs.

Where RSA magnitude has been used to indicate vagal tone, the findings of longitudinal investigations into the effects of training are equivocal. Two studies have revealed no statistically significant change in previously sedentary adults (Maciel *et al.* 1985; Seals and Chase 1989) although in the latter study an increased SDRR in 8 of 11 subjects ($P < 0.001$) was observed. RSA was found to increase post-training in a small ($n = 9$) group of track athletes (De Meersman 1992) although the 8-week training programme they undertook involved a large volume (61 km·week) of high intensity (75 - 95% $\dot{V}_{O_2 \max}$) daily training. Again using high intensity (85% $\dot{V}_{O_2 \max}$) exercise, Al-Ani *et al.* (1996) found significant increases in RSA magnitude after only 6-weeks of aerobic training (25 min·day). Such increases in RSA amplitude may be evidence of a dose response to training intensity.

The use of spectral analysis to monitor longitudinal changes has also provided equivocal results. Many studies show no significant alterations in HRV measures despite an increase in $\dot{V}_{O_2 \max}$, often accompanied by decreased HR (Boutcher

and Stein 1995; Davy *et al.* 1997; Bonaduce *et al.* 1998; Loimaala *et al.* 2000; Myslivecek *et al.* 2002; Perini *et al.* 2002).

4.3.1. *Studies showing no significant effect of training on HRV.*

Boutcher *et al.* (1995) showed no change in LF or HF in sedentary middle-aged men who exercised at 60% $\dot{V}O_{2\ max}$ three times a week for 8 weeks. Exercising at 70% $\dot{V}O_{2\ max}$ resulted in no statistically significant increases in time or frequency domain measures of HRV in postmenopausal women (Davy *et al.* 1997). Increases in all time and frequency domain HRV measures were evident but none of these reached statistical significance. This again, may be due to violation of the assumptions underlying the statistical test used. From the results given, the standard deviations (of particularly the frequency domain measures) post-training are, on average, double their pre-training levels. This heterogeneity of variances, wide dispersion of scores, and the small sample size ($n = 8$) of this study afford it little statistical power. These factors may have increased the likelihood of a type two error being made.

Following five months of intense training Bonaduce *et al.* (1998) found no changes in HRV measures despite increases in $\dot{V}O_{2\ max}$, and heart geometry. Using 24-hour Holter monitoring, Loimaala *et al.* (2000) found significant increases in $\dot{V}O_{2\ max}$ and reduced HR in previously sedentary adults following five months of running at either 55 or 75% $\dot{V}O_{2\ max}$. These changes were however, not accompanied by any alterations in spectral HRV measures. Perini *et al.* (2000) also found no significant differences in HR or HRV measures at rest after eight weeks of progressive exercise training in elderly males and females despite significant (18%) increases in $\dot{V}O_{2\ max}$. Based on previous findings (Goldsmith *et al.* 1992; Furlan *et al.* 1993; Bonaduce *et al.* 1998) Leicht *et al.* (2003) recruited a calculated sample of ($n = 12$) young and ($n = 12$) mature previously sedentary volunteers and trained them for 16 weeks. There were however, no differences

mid or post-training in either group in any of the spectral measures of HRV reported.

4.3.2. *Studies showing a significant effect of training on HRV.*

In contrast to the above findings a number of studies have reported significant alterations in autonomic balance measured by spectral analysis following exercise interventions (al-Ani *et al.* 1996; Hedelin *et al.* 2000; Melanson and Freedson 2001; Portier *et al.* 2001).

In addition to RSA amplitude analysis, Al-Ani *et al.* (1996) also employed spectral analysis and found highly significant increases in both LF and HF. The authors reported more than a doubling of HF (2051 ± 541 vs. 5131 ± 905 ms²) and an almost three-fold increase in LF (912 ± 147 vs. 2464 ± 621 ms²) resulting in an effect size far in excess of any other reported in the literature. Perhaps more remarkable is that in two subjects, there were even greater changes in HF power but in the opposite direction (8281 ± 706 vs. 2602 ± 813 ms²). The values reported in this study are well in excess of those reported (Camm *et al.* 1996) for the normal population (LF = 1170 ± 416 and HF = 975 ± 203 ms²). Reliability coefficients are not reported and no control group was used in the study. The results of this study should therefore be interpreted with caution.

Schuit *et al.* (1999) exercised previously sedentary, older subjects with 3 x 45-min supervised training sessions at 60-80% $\dot{V}O_{2\max}$ for six months. Subjects in the experimental group showed a small increase in 24-Hour measures of: SDNN, LF and VLF (log units) while the control group (no exercise) showed small decreases in all HRV variables ($P > 0.05$). Changes in SDNN and HF were found to be strongly correlated with changes in average RR interval length. Changes, especially in daytime HRV remained statistically significant when this was controlled for. The changes in the control group were attributed to reduced physical activity due to normal seasonal variation (winter). It is of interest to note

that neither pNN50 or HF, which are typically used as markers of PNS activity, increased in this study.

Conversely, Hedelin *et al.* (2001) found a decrease in LF, when measured in the standing position following, seven months of aerobic training. This decrease was not associated with increased $\dot{V}O_{2\max}$ in the mixed group of athletes studied. The effect of training does not seem to be homogenous as both increase and decreases in $\dot{V}O_{2\max}$ and HRV measures were reported. Portier *et al.* (2001) found decreased LF in standing and supine postures in athletes during an intensive training period when compared with a period of relative rest. When expressed in normalised units LF and HF were decreased and increased respectively during the intensive training period. This significant decrease in the LF:HF ratio when measured supine or standing was interpreted as showing a shift towards parasympathetic dominance

Following 12 weeks of intense aerobic training (70-80% $\dot{V}O_{2\max}$) Melanson *et al.* (2001) found significant increases in RMSSD, pNN50 and HF power (log units) in previously sedentary, adult males when compared with pre-training values and controls. In contrast to other studies the decrease in resting HR observed was not statistically significant. In light of this finding and the lack of a statistically significant relationship between training-induced bradycardia and HF power Melanson *et al.* (2001) concluded that spectral measures of HRV were not good indicators of PNS activity.

Using CGSA, (Myslivecek *et al.* 2002) it was found that 12 weeks of walking training reduced SNS indicator values in pre- and post-menopausal females. This was only true when measured in the free-standing position and no differences were found in SNS indicator, PNS indicator or TP when subjects were assessed in either the sitting or left lateral decubitus position.

Iwasaki *et al.* (2003) employed a very large training stimulus in a previously sedentary mixed group of subjects over 12 months. Spectral measures showed significant improvement in LF power at 3, 6, 9 and 12 months compared with baseline. HF almost doubled from 763 ± 233 to 1318 ± 246 at three months but this difference failed to reach statistical significance probably due to the small ($n = 11$) sample size. Utilising CGSA, (Carter *et al.* 2003) positive, overall group changes in TP, fractal power,* HF, PNS and SNS indicators were found following 12 weeks of periodised training. When subjects were divided into four groups by sex and age ($n = 6$ per group) young females showed increased TP, fractal power, HF and PNS indicator values. Older females showed increases in SDNN, HF, and TP. It should be noted that these results represent a seven significant findings from a total of 32 comparisons made without the use of a correction factor for alpha.

4.3.3. *Summary.*

The data are clearly equivocal and seems to be dependent on the measures used. Poor application of statistical analyses and low statistical power may in part, be responsible for some of the nonsignificant results published. This is especially true when raw units are reported. These are commonly skewed, and have wide dispersion, the former prohibits the use of parametric statistics, the latter reduces statistical power. When normalised units or raw units are suitably transformed training is commonly found to produce significant alterations in spectral power.

4.4. **Heart rate variability during exercise.**

The 'research relationship' between exercise and HRV is both complex and circular in nature. HRV has been used as a non-invasive measure of autonomic balance during exercise. Conversely, as the vagal withdrawal and sympathetic activation which accompany rhythmic exercise are well documented (Astrand *et al.* 1987) exercise has been used as a tool with which to gain insight into the

origins of HRV. This is particularly true of the rhythmic HR oscillations the LF and HF bands.

This circular relationship makes the discussion of HRV during exercise a complex topic. In addition to this, a number of exercise protocols have been used. Rhythmic exercise has been studied using either a single intensity of steady state exercise (Arai *et al.* 1989; Dixon *et al.* 1992; Kamath and Fallen 1993; Myslivecek *et al.* 2002), a number of steady state intensities, (Perini *et al.* 1990; Breuer *et al.* 1993; Gregoire *et al.* 1996; Macor *et al.* 1996; Piepoli *et al.* 1996; Tulppo *et al.* 1996; Perini *et al.* 2000; Perini *et al.* 2002), incremental stages, (Yamamoto *et al.* 1992; Casadei *et al.* 1995), ramping (Yamamoto and Hughson 1991; Nakamura *et al.* 1993; Tulppo *et al.* 1998) and even simulated altitude (Yamamoto *et al.* 1996). Static, isometric exercise has also been used (Taylor *et al.* 1995; Raymond *et al.* 1997; Iellamo *et al.* 1999).

Research in this area can be divided into two distinct methodologies, studies that have used general spectral analysis (GSA) and those which have used coarse grained spectral analysis (CGSA). The former uses either FFT or autoregressive modelling to decompose the power spectrum from the original time series. Although these methods can create the same measures, values may differ (Fagard 2001). CGSA can produce measures, which provide similar insights into autonomic function to those gained from GSA. Although some of these measures are analogous to those extracted by GSA, values are not always comparable. The findings of studies using CGSA are therefore discussed separately.

4.4.1. *The HRV response to exercise – results from studies using general spectral analysis.*

Despite the disparity in methodologies used there are several patterns that emerge from studying HRV during exercise. Firstly, when expressed in either the frequency domain (as TP) or in the time domain (as SDNN), HRV is greatly

reduced during exercise (Arai *et al.* 1989; Furlan *et al.* 1990; Perini *et al.* 1990; Dixon *et al.* 1992; Breuer *et al.* 1993; Casadei *et al.* 1995; Macor *et al.* 1996; Perini *et al.* 2000). Where incremental exercise has been used, it is clear that the decrease in HRV occurs as a function of exercise intensity (Perini *et al.* 1990; Breuer *et al.* 1993; Casadei *et al.* 1995; Tulppo *et al.* 1996; Tulppo *et al.* 1998) and that the decrease in variability may quickly reach the limit of resolution of some analysis systems (Rimoldi *et al.* 1990; Pagani *et al.* 1995). Methodologically this means time and frequency domain measures of HRV can be difficult to interpret, especially at higher exercise intensities.

The acquisition of TP from short-term HRV measures gives little insight into the autonomic function of the heart at rest or during exercise and measures should be interpreted with caution (Camm *et al.* 1996). Of more interest are the changes seen in the LF and HF power spectra. It would be expected that HF would decrease during exercise as a function of intensity and that LF would increase due to the proposed association of these measures with PNS and SNS activity respectively (Pagani *et al.* 1986). This is however, not the case.

4.4.1.1. Spectral measures of HRV during exercise.

When expressed in raw unit (msec^2) both LF and HF decrease exponentially as a function of exercise intensity (Arai *et al.* 1989; Perini *et al.* 1990; Rimoldi *et al.* 1992; Casadei *et al.* 1995; Tulppo *et al.* 1996; Tulppo *et al.* 1998; Perini *et al.* 2000; Perini *et al.* 2002). Little therefore, can be gained from the study of these frequency components when expressed this way. In order to gain insight into the changes in autonomic control associated with exercise, researchers have used one or more of the following:

- normalised units,
- the expression of LF and HF as percentages,
- the LF:HF ratio.

4.4.1.2. Changes in LF% and HF% during exercise.

As most exercise is undertaken in the upright position, exercise HRV measures should only be compared with resting measures made in the standing position to control for the orthostatic stress induced by assuming this posture (Pagani *et al.* 1986; Bernardi *et al.* 1996).

During a single bout of steady state (50% $\dot{V}O_{2\max}$) exercise it was found that LF% was greater when compared with measurements made when the subject laid supine, but unchanged when compared with standing values (Dixon *et al.* 1992; Kamath and Fallen 1993). HF% was also unchanged compared with standing values.

Using multiple, relative exercise intensities, LF% and HF% were found to be similar to standing values at 50 W but then to fall rapidly at 100 W and 150 W (Perini *et al.* 1990). Similarly LF% has been shown to increase slightly whereas there is a slight decrease in HF% at 110 W. These values then rapidly decrease in an almost inverse linear pattern with exercise intensity up to 221 W (Casadei *et al.* 1995). In healthy, older subjects a similar decline in LF% has been demonstrated (Perini *et al.* 2000), but this was found to be accompanied by an almost linear reduction in HF% power over exercise intensities equal to 5 – 25 ml.kg⁻¹.min⁻¹.

Macor *et al.* (1995) studied subjects while resting and cycling in the supine position at 20, 40 and 60% of $\dot{V}O_{2\max}$. In contrast to previous findings it was found that LF% increased between rest and low intensity exercise, it then decreased but remained elevated compared with resting values. In common with previous findings HF% was found to be drastically reduced (25 times) at 20% $\dot{V}O_{2\max}$ and by over a 100 times at 60% $\dot{V}O_{2\max}$.

Using LF% and HF% to study autonomic balance during exercise, there is some evidence to suggest a slight augmentation of LF%, accompanied by a reduction

in HF% at lower intensities. As exercise intensity increases, both these values drop dramatically, indicating a greater proportion of the total spectral power to be in the VLF waveband (Perini *et al.* 1990; Casadei *et al.* 1995). A reduction in HF% would be expected, as HF is predominantly, vagally mediated. Such findings also support the partial parasympathetic mediation of LF. This explains in part, the observed decrease in both these measures with increased exercise intensities. Such findings do not, however, support the sympathetic evolution of LF. In fact, a complete removal of LF from the power spectrum at higher exercise intensities has been reported (Perini *et al.* 2000).

4.4.1.3. LFnu, HFnu and LF:HF ratio during exercise.

To exclude the influence of the VLF waveband on the study of autonomic balance during exercise several authors have presented LF and HF in normalised units and by using the LF:HF ratio. As the balance between LF (nu) and HF (nu) is quantitatively identical to the LF:HF ratio, for simplicity only the latter will be discussed here.

The use of the LF:HF ratio as a measure of sympathovagal balance is supported by evidence from orthostatic stress tests and pharmacological manipulation (Pagani *et al.* 1986; Furlan 1987; Montano *et al.* 1994; Pagani *et al.* 1997; van de Borne *et al.* 1997). The use of active standing, passive tilting and pharmacological sympathetic stimulation are known to increase the LF:HF ratio. This has led to the use of the LF:HF ratio as a marker of sympathetic activation. If this were the case, during exercise, one would expect the LF:HF ratio to increase as a function of exercise intensity.

During a single steady-state bout of exercise LF:HF was found to be unchanged compared with standing values (Kamath *et al.* 1991). During incremental exercise a number of authors have found the LF:HF ratio to be increased at lower intensities and to then decline with increasing exercise intensity (Arai *et al.* 1989; Rimoldi *et al.* 1992; Breuer *et al.* 1993; Casadei *et al.* 1995; Tulppo *et al.* 1996).

This pattern of change is not however, universally reported. During incremental exercise Perini *et al.* (1990) found LF:HF ratio decreased steadily up to 50% $\dot{V}O_{2\max}$. It then increased, although not significantly at 70% $\dot{V}O_{2\max}$. Findings from the same laboratory using older subjects (Perini *et al.* 2000) showed LF:HF ratio to remain unchanged during low intensity exercise before decreasing at higher intensities. The authors cited a largely decreased activity in the LF power spectrum to be the reason for this.

To examine further the relationship between LF:HF ratio, exercise and sympathetic activation Breuer *et al.* (1994) assessed spectral measures of HRV, plasma catecholamines and lactate levels simultaneously. Subjects undertook two separate exercise protocols, differences in the above measures were assessed when subjects went from rest (sitting on a cycle ergometer) to pedalling at a rate which elicited a HR of either 100 or 150 BPM. In absolute units, LF and HF decreased dramatically at both intensities when compared with resting values. The LF:HF ratio at 100 BPM was found to increase from 2.0 at rest to 3.3. At 150 BPM blood lactate and catecholamine levels were all significantly increased from rest. However, in agreement with previous studies, the LF:HF ratio was significantly reduced. In addition to exercise, Breuer *et al.* (1994) also used sequential infusion of atropine and catecholamines to simulate the vagal withdrawal and sympathetic stimulation that is known to accompany exercise. Increased HR via atropine infusion was associated with a significant decrease in power in both the LF and HF bands. During the subsequent infusion of catecholamines, HR increased significantly and all HRV disappeared completely.

These findings lend support to the notion that vagal activity modulates both LF and HF power. They do not however support the use of LF or therefore LF:HF ratio as markers of sympathetic activation. The pharmacological findings and lack of association between catecholamine levels and LF, or LF:HF ratio during exercise question the usefulness of HRV under such conditions. The authors

concluded that the use of HRV may not be justified during exercise above the anaerobic threshold, especially as a tool to assess PNS and SNS activity. This viewpoint is supported by findings from an alternative method of spectral analysis (Yamamoto and Hughson 1991; Yamamoto *et al.* 1992) which will be discussed in the next section.

4.4.1.4. The role of methodological heterogeneity in the disparity of results.

Prior to discussion of HRV analysis via CGSA it is useful to quantify the role of methodological variation to the heterogeneity of results from empirical investigations during exercise. Casadei *et al.* (1994) assessed HRV during incremental exercise using GSA and reported their findings in three ways used in previous investigations.

When reported in raw units the expected decrease in TP, was evident. LF increased non-significantly from rest to 110 W but then decreased until it disappeared totally at 221 W. HF was dramatically reduced at 110 W and then remained unchanged until the end of exercise. Using normalised units it was found that LF (nu) initially increased (110 W) but then fell linearly until it had disappeared at 221W. Conversely, HF (nu) initially decreased but then increased with exercise intensity until it accounted for 80.4% of spectral power at 221W. When expressed as a percentage of total RR variance (including the VLF component) it was found that LF% initially increased but then disappeared and HF% remained unchanged throughout the protocol. VLF% remained unchanged from rest to 147W (accounting for less than 30% of TP) but then increased dramatically until it accounted for more than 65% of TP.

The authors stated that the use of LF and HF in raw units and in normalised units during exercise may be misleading. The former is modulated very strongly by the reduction in total spectral power which accompanies the reduced RR interval

associated with exercise. The increase in HF (nu) was attributed to non-neural factors such as stretch and atrial node activity.

It was concluded that spectral analysis of RR interval variability is unable to provide adequate assessment of PNS or SNS activity. The decrease in power in the LF and HF bands has been interpreted as a withdrawal of vagal activity. This, therefore, gives no additional information to that which could be gained from analysis of a more simple measure such as SDNN. The authors also claimed that the use of normalised units may be misleading due to the alternative genesis of HF oscillations during higher intensity exercise. Likewise, it was concluded that RR interval oscillations in the LF band cannot reflect sympathetic activation as they disappear at higher workloads where adrenergic activity is known to be increased.

4.4.1.5. Reasons for the unexpected behaviour of spectral measures during exercise.

The decrease in LF:HF observed at higher exercise intensities is largely due to increased HF%. There is evidence that the origin of some HF oscillations during exercise may be non-neural in nature (Bernardi *et al.* 1990). During incremental exercise, sedentary subjects displayed the expected pattern of changes in LFnu (initial increase followed by a decrease as exercise intensity was augmented) and HFnu (initial reduction followed by increase). A similar but more pronounced effect was found in highly trained cyclists. However, in cardiac transplant recipients no evidence of an LF peak was found at rest or during exercise. A small amount of power was detected in the HF frequency at rest (1.17 ms^2) which increased significantly with exercise at 25 W (7.16 ms^2). Although this value decreased at 50 W (3.44 ms^2) it was still significantly raised compared with resting values.

Casadei *et al.* (1996) attempted to quantify the non-neural contribution to RSA in an attempt to explain the preservation of HF during exercise by measured HRV

at rest and during exercise in subjects with or without ganglionic blockade (GB). At rest, GB abolished the RR interval fluctuation in the LF band. HF was greatly reduced but was still present and showed a high coherence with the breathing signal and the RR interval. During steady state exercise ($25\% \dot{V}O_{2\max}$) mean RR interval and RR variance were greatly reduced. This moderate level of exercise was accompanied by an increased LF:HF ratio common with increased sympathetic activation and vagal withdrawal. During exercise GB abolished LF and decreased the absolute power of HF but failed to cause a significant change in RSA at rest. It was calculated that at rest, non-neural mechanisms are difficult to identify but during exercise the proportion of HF power due to non-neural mechanisms was increased by 32% (range 17-75%).

It was concluded that RR interval oscillations in the LF band are entirely neurally mediated but that non-neural mechanisms contribute to the RSA in young healthy subjects. Although the contribution of non-neural factors at rest is negligible, during moderate intensity exercise may account for up to one third of RSA amplitude.

Further evidence from the existence of non-neural oscillations comes from power in the HF band in patients with fixed atrial pacing (Lombardi *et al.* 1995). The possible origins of these non-neural sources of HF oscillation include mechanical stretching of the SA node (Bolter 1994). It is known that changes in intramural pressure similar to breathing can cause cyclic acetylcholine release, although evidence of persistent HF oscillation under muscarinic blockade suggests a more direct effect such as increased spontaneous depolarisation due to increased Cl⁻ conductance (Kohl *et al.* 1994).

Regardless of the origins of these oscillations, their presence in terms of the use of HRV during exercise, means that despite the greatly reduced RR variance during exercise, a degree of HF variability will be preserved. This non-neural power input to the HF band will serve to grossly overestimate the level of cardiac

vagal activity. This will be especially true when HF%, HFnu or LF:HF ratio are reported. These findings explain the perhaps unexpected behaviour (increase) of LF:HF ratio at higher exercise intensities. It may therefore be concluded that although LF:HF ratio may serve as a reliable estimate of cardiac sympathovagal balance at rest, it may severely overestimate cardiac vagal tone during exercise and any pathological condition where RR variance is known to be reduced (Casadei *et al.* 1996).

4.4.1.6. The use of alternative exercise protocols to investigate the behaviour of spectral measures during exercise.

All the data on which the above conclusions are made are all based on upright exercise. Using recumbent cycling, Macor *et al.* (1995) demonstrated changes in HRV coherent with the notion that LF and HF represent sympathetic and parasympathetic activity respectively. To elucidate further the effects of increased thoracic blood volume and altered orthostatic load, Perini *et al.* (1998) observed the effect of water immersion on HRV responses at rest and during exercise. Resting and incremental exercise HRV measures were made with subjects either in air or immersed in water (head out). At rest it was found that: HF, LF and VLF demonstrated a three-fold increase when expressed in raw units. Power in the HF band expressed as HF(nu) and HF% was increased whereas LF(nu) LF% and VLF% remained unchanged..

During exercise, alterations in HRV measures were similar to those shown previously (Casadei *et al.* 1994). LF% increased initially and was then reduced and a converse effect of exercise on HF% was observed and VLF% increased with exercise intensity. These changes were independent of whether the exercise took place in air or water.

The increase in HF during resting immersion was attributed to a greater respiratory effort being responsible for an enhanced vagal tone. This claim is based on evidence that the vagal nerves are involved with transmission of

fluctuations in the entire frequency bandwidth (Pomeranz *et al.* 1985; Saul *et al.* 1990).

4.4.1.7. Alternative data treatment.

Alternative data manipulation techniques have also been suggested in an attempt to rectify the unexpected behaviours of standard HRV measures and ratios (Warren *et al.* 1997). By using GSA during several intensities of steady state exercise these authors were able to assess the behaviour of HF/TP and LF/TP. These were derived from a number of different HF and LF bands as measures of parasympathetic and sympathetic activity respectively. LF, HF and LF:HF from traditional frequency bands were also assessed. Warren *et al.* (1997) also attempted to validate new and existing indices using muscarinic, β -receptor and combined blockade at rest and during exercise. They found that HF (0.1-1.0 Hz) provided a reliable index of vagal activity during exercise. Its behaviour corroborated HR response and was supported by pharmacological data. An equivocal index of sympathetic activity was provided by LF/TP (0.004-0.1/0.004-1.0 Hz). If corroborated by further findings these results could be of great importance in providing information regarding autonomic fluctuations during exercise.

4.4.1.8. Summary of findings using GSA during exercise.

In conclusion, several authors (Perini *et al.* 1990; Casadei *et al.* 1995; Casadei *et al.* 1996; Perini *et al.* 2000; Perini *et al.* 2002) agree that HRV is a useful measure for assessing the resting autonomic balance but that its use during exercise is limited. It is clear that the correct treatment and representation of data is crucial as certain representations of findings may be misleading. It seems that insight into autonomic control during exercise from traditional GSA measures of HRV may be limited to lighter exercise intensities. Few studies have attempted to validate findings with autonomic blockade. Where this has been done during exercise (Warren *et al.* 1997) proposed new data treatment techniques show

promise in the ability of HRV to provide information regarding exercise autonomic function. Conversely to this, exercise itself has provided researchers with insight into the origins, and therefore uses, of HRV measures derived from this method.

4.4.2. *Heart rate variability response to exercise: findings from studies using coarse graining spectral analysis.*

In light of such equivocal findings, research concerning HRV during exercise has been carried out using further alternative treatments of the RR interval data. One such method is CGSA. This method has been used to examine both the linear and non-linear (fractal or chaotic) features of RR interval data. Although a full review of fractal dynamics is beyond the scope of this chapter, where necessary, basic explanations of the mathematical methods used will be given. For a full review the reader is directed to the excellent technical reviews which already exist in this area (Hagerman *et al.* 1996)

4.4.3. *HRV during exercise: Studies using CGSA.*

In a series of papers Yamamoto *et al.* (1991, 1991b, 1992) and Nakamura *et al.* (1992) first introduced the concept of CGSA, then used the method to examine the activity of the PNS and SNS during exercise. CGSA provides improved resolution of short-term HRV spectra by removing the fractal component* known to exist in HR data (Saul *et al.* 1988), from the harmonic components*. This separation facilitates enhanced analysis of the latter. The fractal component is a series of oscillations occurring at differing frequencies. Under GSA these oscillations constitute the VLF component of HRV. Fourier analysis of RR interval data has shown the existence of fractal 'noise' at frequencies from 0.00003-0.01Hz (10 hours - 10 seconds) and it is believed that this noise may affect assessment of sympathovagal balance (Yamamoto and Hughson 1991).

Due to the removal of parts of the spectrum the high and low frequency bands calculated by CGSA are not quantitatively similar to the LF and HF derived from GSA. The use of this terminology would therefore be incorrect. Using CGSA the low and high frequency bands are commonly reported as Low and High respectively, although this is not universal. The LF:HF ratio from GSA is therefore replaced in CGSA by the Low:High ratio. Originally High was referred to as the PNS indicator. Both Low and Low/High were referred to as the SNS indicator (Yamamoto and Hughson 1991; Yamamoto *et al.* 1991). Confusingly, some authors have calculated the additional ratio of High/Total Power and also called this the PNS indicator (Gregoire *et al.* 1996; Amara and Wolfe 1998; Myslivecek *et al.* 2002).

4.4.3.1. Harmonic oscillations during exercise measured by CGSA.

In their introductory paper to CGSA (Yamamoto and Hughson 1991) gave an illustration of the effects of ramped ($15 \text{ W}\cdot\text{min}^{-1}$) exercise on Low and High spectral peaks. It was found that both Low and High spectral peaks declined as exercise intensity increased. Both spectral peaks were eliminated by the time 'moderate' exercise intensity was reached. From this it would seem that CGSA would be of little or no use in the study of exercise HRV.

The same authors (Yamamoto *et al.* 1991b) performed GSA and CGSA on RR data from six, 14 min, steady-state exercise stages and found the PNS indicator declined steadily from rest to 60% of ventilatory threshold (T_{vent}) and that the SNS indicator increased only above the T_{vent} . The authors stated that this gave an indication of the expected autonomic response to exercise. The authors seem to largely ignore two facts concerning these data. Firstly, these data contradict their own previous findings (Yamamoto and Hughson 1991). Secondly, and perhaps more importantly, the data from GSA differ greatly from those published previously (Arai *et al.* 1989). The authors also fail to mention the great similarities in patterns of data using GSA or CGSA. In terms of the Low:High

ratio the large increase (1.77 – 6.19) cited as a major finding using CGSA is mirrored by a change of similar magnitude shown by GSA (4.0 – 13.5 approximately). This latter finding failed to reach statistical significance, probably due to the large interindividual differences in changes in Low/High from 100 – 110% T_{vent} . The LF:HF ratio data computed from GSA in this study show changes that are diametrically opposed to those reported in studies carried out before (Arai *et al.* 1989; Perini *et al.* 1990) and after (Breuer *et al.* 1993; Casadei *et al.* 1995; Tulppo *et al.* 1996) in which the LF:HF ratio was calculated using the same analyses. On this basis the author's claims that CGSA may be a more sensitive tool for use during exercise may be based on spurious data and therefore, seriously flawed.

These authors further investigated the response of GGSA derived HRV, at T_{vent} (Yamamoto *et al.* 1992) this time using a ramping protocol (2 W·min). Again a steady decrease in PNS indicator up to T_{vent} was accompanied by an increase in SNS indicator above T_{vent} . Although the authors reported these findings as evidence of a large increase in SNS activity at T_{vent} these results should also be treated with caution for a number of methodological reasons.

Yamamoto *et al.* (1992) make no mention of the large reduction in SNS indicator, clearly evident in their results, which occurred at the final work rate, 40 W above T_{vent} . Additionally, values for the PNS indicator can be seen to increase from 30 W to 40 W above T_{vent} . Methodologically, none of the results were acquired from steady state exercise. The ramping protocol used creates only 'quasi-steady state' conditions. The authors attempted to control for this by removing linear trends in the data. The authors gave no data concerning the success of this delineation and it is clear from the results, that many of the responses at lower intensities and at T_{vent} were non-linear in nature. The authors note the use of such a protocol is unlikely to demonstrate T_{vent} and the normal respiratory indicators of this phenomenon indeed show no evidence of the normal changes associated with it. Warren *et al.* (1997) make the point that certain RR interval treatments give results that may be coincidental with established theories of

autonomic response. Although they do not make direct reference to CGSA, their further observation that exercise HRV has not been validated by autonomic blockade or microneurographic studies during exercise is indeed true with regards to CGSA. Data regarding validation of the harmonic components at rest using pharmacological blockade (Tulppo *et al.* 2001) and from tilt, in patients with autonomic failure (Blaber *et al.* 1996) provide equivocal results. Lack of agreement between CGSA derived measures of central sympathetic outflow and direct microneurographic estimates has also been shown (Notarius *et al.* 1999).

Using CGSA during exercise Nakamura *et al.* (1993) reproduced previously obtained results (Yamamoto *et al.* 1992) using an identical protocol. Nakamura *et al.* (1993) concentrated their investigation further into changes in the fractal component of HRV. This had, until now, been largely ignored. The fractal component was assessed by plotting the log frequency vs. log power with β estimated as the slope of the linear regression of this plot. This plot is representative of the complexity of a time series with values $1 < \beta < 3$ indicating a fractal nature. The more complex the time series the higher the value of β (1-3). Mathematically, β is representative of the number of independent oscillators responsible for the generation of any given time series (Yamamoto *et al.* 1992). Physiologically, it is claimed that β is representative of the number of inputs which affect the cardiovascular control centres and therefore, control HR (Goldberger 1990).

Nakamura *et al.* (1993) found that β approximated 3 during mild exercise and decreased linearly during moderate to high exercise intensities. This occurred simultaneously with the decrease in PNS and increase in SNS indicators observed at high intensity exercise, and β was reduced to <2 indicating a single dominant controller to be impinging on HR. As this intensity of exercise coincided with a marked lactacidaemia the authors hypothesised that the arterial chemoreceptor afferent may be that input.

Further investigation into the fractal nature of the VLF component of RR interval data (Hagerman *et al.* 1996) confirmed the chaotic nature of the RR interval

oscillations in the VLF band by calculating the Lyapunov exponent*. The values obtained were in agreement with previous work (Nakamura *et al.* 1993). Using the $1/f^x$ slope* it was found that the complexity of the fractal component was reduced but not removed with increasing exercise intensity.

Further examination of the non-harmonic component in relation to other stimuli such as beta-adrenergic blockade (Yamamoto and Hughson 1994; Yamamoto *et al.* 1995; Tulppo *et al.* 2001; Perkiomaki *et al.* 2002) sinus arrhythmia (Yamamoto *et al.* 1995) simulated altitude (Yamamoto *et al.* 1996) has continued. However, discussion of these findings is beyond the scope of this paper.

Discussion of the use of the harmonic SNS and PNS indicators derived from CGSA is, however, pertinent. Using the method of Yamamoto *et al.* (1991), changes in the PNS and SNS indicators similar to those previously reported have been observed during absolute exercise intensities of 50 W and 100 W (Gregoire *et al.* 1996) and at a single relative exercise intensity of 40% HR_{max} (Myslivecek *et al.* 2002). The responses noted were found to differ little in groups defined by age, gender and habitual physical activity levels (Myslivecek *et al.* 2002).

4.4.3.2. Summary of findings from CGSA.

To conclude, it seems that CGSA may be a more useful tool with which to observe the changes in autonomic function that accompany exercise. In contrast to the findings derived from GSA, it is commonly found that the expected decrease in PNS indicator during low intensity exercise is followed by an increase in the SNS indicator at higher intensities. This pattern, followed by an abrupt increase in the SNS indicator at or around the T_{vent} is analogous to the known sympathetic response observed during incremental exercise using invasive methods (Wallin *et al.* 1992; Kingwell *et al.* 1994). However, the harmonic components derived from this method during exercise have not yet been fully validated. Until there is concurrent microneurographic or

pharmacologic data regarding PNS and SNS indicators, responses to exercise results from CGSA exercise studies should be treated with caution.

In addition to the information produced by the PNS and SNS indicators, the ability of CGSA to separate the harmonic and fractal components of the heart signal provides further insight into the nature of autonomic control of HR. The fractal component increases as a percentage of the total spectral power with increasing exercise intensity but the component's chaotic nature at rest is simplified, approaching unity during high intensity exercise. The parallels between this harmonising effect and the changes in this component at time of death warrant further investigation.

4.5. Cross-sectional and longitudinal studies examining differences in the HRV response to exercise.

Few studies have attempted to compare the HRV response to exercise between groups of subject. This is in part, due to the unresolved methodological issues which accompany the use of HRV (particularly from GSA) during exercise. In the existing data, findings are equivocal.

Using autoregressive spectral analysis during upright exercise (50% max workload) Dixon *et al.* (1992) found no difference in autonomic balance during exercise between athletes and controls despite significant differences at rest. Similar findings were produced during recumbent cycling (Macor *et al.* 1996) at 20, 40 and 50% $\dot{V}O_{2\ max}$.

Using incremental, upright cycling Tulppo *et al.* (1998) found that HF power during exercise from 50 – 100 W was significantly higher in an 'average' ($\dot{V}O_{2\ peak}$ 51 ± 4 ml·kg⁻¹·min⁻¹) compared with a 'poor' fitness group ($\dot{V}O_{2\ peak}$ 34 ± 3 ml·kg⁻¹·min⁻¹). This difference was not evident at rest and disappeared at higher exercise intensities. Longitudinally, Perini *et al.* (2002) found no differences in LF(nu) or HF(nu) response to exercise after an 8 wk aerobic training programme.

Using CGSA, Gregoire *et al.* (1996) found that young, trained subjects showed greater preservation of TP when cycling at 100 W compared with untrained controls. This increase was also accompanied by a greater SDRR. There were no differences in the SNS or PNS indicators in young subjects either at rest or during exercise. The same method was used to monitor longitudinal changes due to endurance training (Myslivecek *et al.* 2002). During exercise (40% HR_{max}) it was found that PNS and SNS indicators were significantly increased and decreased respectively in the exercise group post-training compared with baseline values and changes in controls.

Cross-sectional findings from GSA are clearly equivocal although there may be some evidence of preserved parasympathetic tone during low to moderate intensity exercise. Perini *et al.* (2001) failed to show any longitudinal alteration in HRV measures during exercise due to training. It should be noted that no statistically significant differences in resting HRV measures were detected in this study either.

4.5.1. *The usefulness of HRV measures during exercise.*

It seems clear that if GSA is to be used to investigate differences in autonomic control during exercise that it may only serve to provide researchers with additional, useful information at lower exercise intensities.

Where CGSA has been used, clearer distinctions between trained and untrained groups and pre- and post-training conditions have been demonstrated. This method is not without its limitations. Gregoire *et al.* (1996) were unable to process data from higher exercise intensities due to low signal:noise ratios and loss of spectral power. Additionally it has been noted (Amara and Wolfe 1998; Myslivecek *et al.* 2002) that the PNS indicator shows large intra- and intersubject variation. This measure is highly sensitive to small fluctuation in HF spectral power. During exercise, where TP is greatly reduced this may be problematic

when making intra- and intergroup comparisons. This phenomenon was illustrated in the findings of Myslivecek *et al.* (2002). Where pre- and post-training comparisons were made in a heterogeneous group (pre- and post-menopausal females) no significant differences were found. This was attributed to the wide spread of scores for the PNS indicator.

In section 3.3.1 the findings of Leicht *et al.* (2003) were discussed. Analysis of RR interval by GSA failed to show any differences in HRV measures post-training at rest. During walking exercise, (2 km per hour) LF and TP were found to be significantly raised when compared with pre-training values in mature subjects. There was also improved preservation of TP at a work rate corresponding to 50% HR_{max}.

4.6. Summary

Resting HRV analysis via simple RSA amplitude, time series and the more complex spectral measures is capable of detecting differences in indicators of autonomic balance between athletes and controls. When 'trained' and 'untrained' individuals are compared the data are equivocal. This is possibly due to unclear definitions of these terms and group allocation criteria.

Longitudinal studies are also equivocal in their findings. This may be due in part to research design, such as insufficient training stimulus. The heterogeneity of findings from similar study designs suggests a simpler methodological or statistical issue may be responsible

Both study designs show clear examples of a fundamental problem in the use of HRV, namely the large interindividual differences which exist in the measures. The large spreads of scores commonly reported create overlap between group dispersions. This in turn, makes the detection of significant differences between group means difficult. Methodologically, it seems that researchers are more likely to detect differences in raw units when data undergo suitable

transformation. This process can normalise the frequently skewed distributions and allow the application of parametric statistical analyses. In terms of spectral measures, the use of normalised units or the analogous measure %TP both serve to enhance the statistical power associated with given sample sizes. It seems that HRV measures may give clear indications of both cross-sectional and longitudinal differences in autonomic function if the data are treated and analysed carefully.

During exercise the large reductions in spectral power observed are a fundamental problem. Although the use of normalised units and the LF:HF ratio to eliminate this difficulty has been employed, traditional measures from GSA do not reflect the known patterns of PNS withdrawal and SNS activation with increasing exercise intensity although alternative methods are available.

There are also several methodological problems associated with the assumptions underlying the use of FFT on RR interval data, namely recording duration and tachogram stationarity. In spite of this, there is evidence to suggest that GSA may be able to provide information regarding autonomic control during low-to-moderate exercise intensity. This information may add to that gained from resting HRV measures. Clarification of the behaviour of the HF and LF components during incremental exercise is also needed.

With the use of the GGSA method, a clear illustration of PNS withdrawal and SNS activation during exercise can be shown. In addition, more information regarding the nature of HR control may be gleaned from analysis of the fractal or non-harmonic component of the RR interval data. This area warrants further investigation and its application to the study of group differences in trained vs. untrained and longitudinal changes in autonomic control due to exercise is therefore recommended. Of further interest would be the comparison of GSA and CGSA measures derived from the same RR interval data. In the literature this is limited to a single study. In this study, the changes in LF and HF differ from all previous and subsequent studies.

From the limited data where CGSA has been utilised to study group differences and changes, the problem of sensitivity has been raised. This problem may necessitate the use of more homogenous groups or larger sample sizes from those of previous studies in order to show clear differences in autonomic function using CGSA.

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CHAPTER 5. DIFFERENCES IN RESTING MEASURES OF HEART RATE VARIABILITY IN FIT AND UNFIT SUBJECTS AS DEFINED BY $\dot{V}O_2$ PEAK.

Abstract.

Numerous studies have attempted to show differences in HRV measures between athletes and control subjects, between active and inactive individuals, and between subjects categorised as fit or unfit with mixed results. The aim of this chapter was to identify whether significant differences in resting HRV measures existed between subjects classed as fit or unfit using the objective measurement of $\dot{V}O_2$ peak.

Resting, five minute HRV measurements were made in 29 volunteers (20 males, median age 39, range 19 – 63 years and nine females, median age 35, range 19 – 56 years). These subjects then completed an incremental treadmill test to $\dot{V}O_2$ peak. Subjects were divided into the fitness categories of high fitness (HI) and low fitness (LO). Standard time and frequency domain HRV measures were then compared between groups.

Where necessary, data were transformed to allow application of parametric analyses. No significant differences were found in any HRV measures between groups using parametric analyses. The same comparison was made using analysis of covariance where appropriate and also with non-parametric equivalents. Still, no significant differences were found.

In conclusion, it appears that differences in HRV measures between fit and unfit subjects are not statistically significant and that differences in statistical analyses between studies are not the cause of the heterogeneity of results reported in the literature.

5.1. Introduction

Several studies have compared measures of heart rate variability (HRV) between subject groups based on fitness levels. Results have been heterogeneous in nature, reporting both differences and similarities between such groups.

The methodologies used to allocate subjects to groups and the classifications of these groups are different between studies. When athletes and sedentary controls are compared studies have frequently shown statistically significant differences in time and frequency domain measures of HRV (Katona *et al.* 1982; Reiling and Seals 1988; Dixon *et al.* 1992; Janssen *et al.* 1993; Puig *et al.* 1993; Sacknoff *et al.* 1994; Jensen-Urstad *et al.* 1997).

This grouping of subjects according to such criteria gives results representative of only a small minority of the population. Elite athletes such as those often used are exceptional often in number of ways. Differences between athletes and sedentary controls are often due to training volumes, types and intensities beyond the reach or capability of the general public.

More generalisable comparisons are that of recreational athletes and controls, active and inactive subjects or the division of a population according to a lifestyle or physiological measures. Studies have compared populations based on self-reported physical activity (Melanson 2000), maximum workload (Migliaro *et al.* 2001) and $\dot{V}O_{2\text{ max}}$ (Shin *et al.* 1997). Evidence of statistically significant differences in HRV measures between these groups has been equivocal. Some studies show large differences while other data fail to reach statistical significance. In certain cases, either no differences are evident at all or the effect is in the opposite direction to that which would be expected.

There may be a number of reasons for the disparity in findings. Long and short-term HRV measurement techniques have both been used. Where short-term measures have been used, sampling durations have varied. The HRV measures

reported have varied between studies. Some studies have focused on HRV measures representative of mainly parasympathetic modulation. This is often in an attempt to provide evidence of increased parasympathetic modulation to explain the resting bradycardia observed in physically active subjects (Janssen *et al.* 1993; Jensen-Urstad *et al.* 1997). On occasion, this has been done at the expense of reporting other HRV measures which may offer additional insight into autonomic differences between groups (Janssen *et al.* 1993).

Statistical treatment of HRV data has also differed between studies. Due to its dynamic nature, HRV data often display a wide kurtosis. Frequency domain data commonly have skewed distributions (Goldsmith *et al.* 1992; Bigger *et al.* 1993). Many studies give no information concerning normality of HRV data. In some cases where measures of central tendency and distribution are given, it is clear that inappropriate statistical analyses have been carried out.

The purposes of the present study were threefold. Firstly, to compare all recommended, short-term HRV measures from two groups of subjects allocated on the basis of $\dot{V}O_{2\ peak}$ derived from maximal exercise testing. Secondly, to use a variety of statistical methods representative of the current literature to assess the role these may play in the heterogeneity of published results. This included identifying and controlling for any confounding variables which may affect the statistical analysis of differences in HRV. Thirdly, to make recommendations on the representation of differences in HRV measures between groups and give estimates of necessary sample sizes to provide adequate statistical power in further studies.

5.2. Methods.

5.2.1. *Subjects.*

Twenty nine university staff and students (20 males median age 39 range 19 - 63) and nine females median age 35 (range 19 - 56) were included in the study. All subjects were healthy, defined as being free from illness at the time of testing. None were known to be taking any medication or have any cardiovascular problems that may have influenced the tests carried out. All subjects volunteered to participate in the study. Written, informed consent was given separately for each part of the study: resting HRV measures, exercise testing and blood lactate testing. Subjects were, therefore, able to participate in the study without consenting to blood sampling if they so chose. Complete blood lactate analysis was only completed on 20 subjects. One subject was excluded from the HRV analysis due to technical failure. Full HRV data during exercise was not collected on a large proportion of the subjects due to low signal:noise ratio. This limitation is further discussed later in the text.

5.2.2. *Equipment.*

5.2.2.1. Heart rate variability measurement.

HRV measures were made simultaneously using two instruments simultaneously; at rest and during exercise. Instrument one was a CardioPerfect ST 2001 HRV module (Cardio Control, Delft, The Netherlands). The system used a standard 12 lead ECG with a sampling frequency of 1000Hz. The sampling time for each HRV analysis was set at 5-min in agreement with current guidelines (Camm *et al.* 1996). The signal was digitised directly and the full ECG trace was shown in real time on a computer screen, this signal was simultaneously stored on the hard drive of a PC (Dell Computers, Texas, USA.) for *post hoc* analysis. Each sampling period was stored as a single patient ECG record. To analyse the variability in RR interval data an automated protocol

within the HRV module software was used. The automated filtering system is adjustable, and a standardised configuration was used which treated the data as described below.

Editing of the raw RR interval data was carried out using an automatic-threshold-detection algorithm. The software rejected RR intervals which differed by more than 20% from the previous interval. This interval was then interpolated with an interval generated on the preceding interval data. The remaining analysis was based on the corrected data file of 'normal' RR intervals. The RR data were then passed through a Hanning type window to remove baseline trends. The RR interval time series was decomposed to the frequency domain via fast Fourier analysis. The resulting power spectrum was divided into the following spectral bands HF (0.15-0.40 Hz) LF (0.04-0.15 Hz) and VLF (0.0033-0.04Hz). In addition to this time domain analysis was also carried out to give the following values RMSSD, SDNN and RR interval.

The second HRV analysis instrument was a VariaCardio (TF5) HRV analysis system (Advanced Medical Diagnostics Group Ltd. Leeds, UK). This system used a purpose built chest-strap with two electrodes, placed either side of the subject's heart. The chest strap sampled at rate of 500 Hz. This signal was then sent to a receiver and digitised. The ECG trace and a graphical representation of RR interval were displayed in real time on the screen of a designated portable computer (IBM Systems USA). The sampling period for each measurement was set at 300 seconds or 300 beats whichever was longer. The instantaneous HR was derived from the identification of QRS complexes which was sampled at a higher rate of 1000 Hz. The RR interval and full ECG data were both stored on the hard disk of the designated portable computer prior for *post hoc* analysis.

Stored RR interval data were then edited automatically by the software. The manufacturers do not give details of the algorithm used to edit the data. To ensure effective editing all data were manually edited by a single researcher. This was done by visually analysing the raw ECG trace and the graphical

representation of the RR interval data given. The researcher rejected intervals which appeared to represent potential artefacts, interference or noise. This filtered data set was then stored on the hard disk of the PC as a separate data file labelled filtered data. All further analysis was carried out on this filtered data set. The RR interval time series were decomposed to the frequency domain via fast Fourier analysis. The resulting power spectrum was divided into the following spectral bands HF (0.15-0.40 Hz) LF (0.04-0.15 Hz). Time domain analysis was also carried out to give the following values mean squared standard deviation (MSSD), SDNN and NN interval. The MSSD was then transformed to the RMSSD manually.

The reasons for the simultaneous use of two HRV systems were twofold. Firstly the dual analysis protocol was used to maximise the possibility of capturing the maximal amount of data. This is because it is known, especially during exercise that changes in the signal-to-noise ratio may prohibit the spectral analysis of RR interval data (Gregoire *et al.* 1996). Secondly, although HRV analysis is now commonplace during exercise there are no data pertaining to the agreement between different analysis systems under such conditions.

5.2.2.2. Respiratory measurements.

The volume of expired air (V_E) and gas exchange measurements were made breath-by-breath, using a Medical Graphics Cardio₂ online breath-by-breath analysis system (Medical Graphics Corporation, St. Paul Minnesota, USA). Automated software from the same company (Breeze Suite) created a full set of nine-panel-plots (Wasserman *et al.* 1999) by which aerobic threshold could be detected. Blood lactate analysis was carried out using a YSI 1500 Sport lactate analyser (Analytical Technologies, Hanford House, UK).

5.2.3. Protocol

5.2.3.1. Assessment of $\dot{V}O_{2\ peak}$ and $CPO_{\ peak}$.

Subjects visited the laboratory on a total of three occasions to undertake different parts of the testing protocol. During visit one $\dot{V}O_{2\ peak}$ and $CPO_{\ peak}$ were determined for each subject. Each subject completed an incremental exercise test (Bruce) on a motor-driven treadmill (Cardio Control, Delft, The Netherlands) to volitional exhaustion. This allowed an estimate of $\dot{V}O_{2\ peak}$ which was chosen in preference to true $\dot{V}O_{2\ max}$. $\dot{V}O_{2\ peak}$ has been found to be simple to measure, enduring standard of aerobic fitness (Mancini *et al.* 2000). It is less physically stressful than $\dot{V}O_{2\ max}$ for the subject and is therefore useful when using volunteers from clinical populations and the general population. Many subjects within these categories frequently have difficulty attaining true $\dot{V}O_{2\ max}$ as defined by current guidelines (ACSM 2000). During the test \dot{V}_E and O_2 and CO_2 exchange were monitored continuously.

After at least 40-min rest the subjects performed a constant maximal workload exercise test on the treadmill. In this instance the researcher manually controlled the speed and gradient of the treadmill to elicit a $\dot{V}O_2$ close to (within 10%) the value achieved during the Bruce test. Cardiac output (CO) was then assessed using the CO_2 rebreathing technique (Defares 1960). Cardiac power output (CPO) was subsequently calculated using CO and blood pressure measured by a hand-held aneroid sphygmomanometer.

Two non-invasive methods are available to assess cardiac output the equilibrium method (Collier 1955) and the exponential method (Defares 1960). The exponential method was chosen as subjects experience significantly fewer adverse side effects compared with the equilibrium measure (Vanhees *et al.* 2000). There are data to support both the validity (Marks *et al.* 1985; Kuji *et al.* 1991) and reliability (Marks *et al.* 1985) of this method. Briefly, during the constant maximal workload test the subject's air supply was changed from room

air to breathing from a bag containing 5% CO₂ and 14% O₂ for 10-20 s. The subject then rebreathed from this bag until a plateau in the increase in CO₂ concentration was observed. The increase in CO₂ and plotting of the CO₂ concentration during the manoeuvre was carried out by an automated protocol within the BreezeSuite software.

5.2.4. *Calculations.*

Cardiac power output was derived from the following equation:

$$\text{CPO} = \text{CO} \times \text{MAP} \times \text{K} \qquad \text{Equation 5-1}$$

Where MAP is mean arterial pressure, CO is cardiac output as derived from the indirect Fick principle via the rebreathing manoeuvre and K is the conversion factor (2.22×10^{-3}).

It was therefore first necessary to calculate mean arterial pressure by

$$\text{MAP} = (\text{SBP} + 2\text{DBP})/3 \qquad \text{Equation 5-2.}$$

The estimation of cardiac output in this manner is based on the indirect Fick principle. The rebreathing manoeuvre gives an estimation of the mixed venous PCO₂. The arterial CO₂ is estimated from the end tidal partial pressure of CO₂. These two values are converted to concentrations automatically by dissociation tables within the MedGraphics software. These values are then substituted into the indirect Fick equation to give a measure of CO. Equation 5-1 is then used to produce CPO and heart rate obtained from successive RR intervals via the 12-lead ECG can also be used to calculate stroke volume.

5.2.5. *Heart rate variability measurement.*

During the second visit, each subject's resting HRV was analysed. In accordance with current guidelines for the capture of RR interval data for HRV analysis subjects attended the laboratory having refrained from eating or smoking for 2

hours. Subjects were also asked to refrain from alcohol or caffeine containing beverages and exercise on the day of the test, and exercise and heavy alcohol consumption on the evening prior to the test. Where subjects had failed to meet the requirements of the protocol an assessment was made on the potential impact of their behaviour on HRV measures and where necessary the test was rescheduled.

Disposable electrodes (*Blue Sensor Medicotest, Olstykke, Denmark*) were placed in the standard configuration for 12-lead ECG and the TF5 chest strap was placed laterally across the subject's heart in accordance with manufacturer's instructions. Subjects were then asked to lie on a bed in a quiet laboratory (18-22 °C). Subjects were asked to relax and the researchers monitored their HR visually until it became stable. Two simultaneous 5-min ECG recordings were then made. Two researchers were required to synchronously start both pieces of equipment. Although some auditory clues may have been available, the subject was purposely not informed of the start of the recording period. At the end of the first recording the subject was asked to stand with feet shoulder width apart with hands placed on the back of a tall stool. Subjects were asked not to 'shuffle' or transfer their weight laterally during the data collection period. A second 5-min ECG recording was made when the subjects HR was deemed to be stable following the change in position.

5.2.6. *Measurement of HRV, blood lactate and CPO during incremental exercise.*

At the third visit to the laboratory, consenting subjects were first required to give a resting blood lactate sample. An arterialised capillary finger prick sample was taken from the index finger of the left hand and the whole blood was then transferred via micropipette to the YSI 1500 Sport Lactate analyser. All data were recorded from the analyser immediately and stored electronically for later analysis.

Subjects were then required to complete three stages of incremental exercise (25, 50 and 75% $\dot{V}O_{2peak}$). During this test ECG was continually monitored and breath-by-breath analysis of expired air was carried out using the techniques already described. The intensity of this exercise was determined from the $\dot{V}O_2$ at each level. When the level of predetermined $\dot{V}O_2$ was reached, a further 5-min ECG sample was taken. During this period, a blood pressure measurement was taken. At the end of 5-min a blood lactate measurement was made using the procedure described previously. At the end of each stage the subjects performed the rebreathing manoeuvre described earlier but with the addition of being instructed to breathe in time with a metronome eliciting a breathing rate of 0.15 Hz. This was done in accordance with the manufacturer's instruction to ensure the adequate rebreathing of the reference gas mixture from the bag.

5.2.7. *Data analysis.*

Data from the resting and supine condition were divided into two groups; high fitness (HI) and low fitness (LO) determined by $\dot{V}O_{2peak}$ values obtained during maximal exercise testing. Chi squared analysis was used to determine whether gender had a significant association with group allocation.

5.2.7.1. Normality of distribution and homogeneity of variances.

Normality of distribution for each HRV measure was assessed for the whole group using a Shapiro-Wilk's statistic. Additionally homogeneity of variances between the LO and HI groups was assessed simultaneously with a spread vs. level Levene's Test. This was undertaken for raw data and where necessary repeated with a power estimation of transformation by natural logarithms (ln). This procedure produced a plot of the natural logs of the interquartile ranges against the natural logs of the medians for all cells. It additionally produced an estimate of the power transformation for achieving equal variances in the cells.

This spread-versus-level plot determines the power for a transformation to stabilise variances across groups.

5.2.7.2. Statistical tests of difference.

Where appropriate, age, height, weight and body mass index were then analysed by independent *t*-test (either raw or log transformed units) to ascertain whether significant differences were evident between the HI and LO groups.

Where significant differences were found the variable was correlated with all measures of HRV to identify the magnitude and similarity of the relationship with any HRV measures. This was done to estimate the influence of this variable on the HRV measure and to estimate the suitability of the variable as a covariate in further analysis. Where no relationships between subject descriptor variables and measures of HRV were found, independent *t*-tests were used to examine differences between the two groups. Where a suitable covariate was identified analysis of covariance (ANCOVA) was used to control for the effect of this variable.

5.3. Results.

The subject characteristics for the LO and HI groups are given in table 5-1. Chi² analysis revealed no significant association between gender and group allocation. Heterogeneity of variances meant $\dot{V}O_{2\text{peak}}$ required natural logarithmic transformation prior to the application of parametric statistical analysis. Following transformation the mean $\dot{V}O_{2\text{peak}}$ in the HI group was significantly greater than the LO group. With the exception of age ($P = 0.06$), other subject characteristics did not differ between groups.

All HRV variables were first assessed using independent *t*-tests (Table 5-2). Age was identified as a possible confounding variable and correlated with all HRV

measures to estimate the strength of its relationship with each. In the LO group, age was found to correlate significantly with HF, HFnu, LFnu and RMSSD. In the HI group, similar relationships with age were only found between HF and RMSSD. Age was therefore entered as a covariate into the analysis of these two variables only.

Table 5-1. Descriptive characteristics of subjects divided by fitness level.

	LO	HI	Independent t-test
$\dot{V}O_{2peak}$ (log units)	3.2 ± 1.4	3.8 ± 1.2	$t = 7.90,$ $P = 0.001$
Age (years)	42.1 ± 14.5	32.1 ± 12.3	$t = 1.96,$ $P = 0.06$
Height (cm)	173.5 ± 8.4	178.0 ± 8.0	$t = 1.38,$ $P = 0.18$
Weight (kg)	78.7 ± 11.0	77.6 ± 12.8	$t = 0.25,$ $P = 0.80$
BMI	26.0 ± 3.0	24.4 ± 2.6	$t = 1.50,$ $P = 0.15$

Values are (mean ± SD) for subject variables. All values are raw units (unless otherwise stated) and independent *t*-test values are based on the assumption of homogenous variances.

Table 5-2. Parametric analysis of heart rate variability between high and low fitness level subjects.

HRV measure	LO	HI	Independent t-test	Analysis of covariance (controlling for age)
TP (ms ²)	3079.0 ± 2707.4	2568.3 ± 1869.2	$t = 0.55,$ $P = 0.57$	
LF(ln)	2.9 ± 0.5	2.9 ± 0.5	$t = 0.07,$ $P = 0.95$	
HF(ln)	2.7 ± 0.7	2.8 ± 0.5	$t = 0.83,$ $P = 0.45$	$F = 2.1,$ $P = 0.16$
LF (nu)	57.9 ± 17.3	53.2 ± 21.4	$t = 0.62,$ $P = 0.54$	
HF (nu)	42.1 ± 17.2	45.7 ± 20.2	$t = 0.49,$ $P = 0.62$	
LF:HF ratio	1.77 ± 1.89	1.9 ± 1.9	$t = 0.19,$ $P = 0.84$	
SDNN (ms)	60.4 ± 22.3	56.5 ± 18.1	$t = 0.50,$ $P = 0.62$	
RMSSD (ln)	3.7 ± 0.7	3.8 ± 0.5	$t = 0.46,$ $P = 0.65$	$F = 0.79,$ $P = 0.38$
RR interval (ms)	961.0 ± 138.8	982.6 ± 107.9	$t = 0.45,$ $P = 0.65$	

Values are (mean ± SD) for subject variables. All values are raw units (unless otherwise stated) and independent *t*-test values are based on the assumption of homogenous variances. Analysis of covariance was used when a suitable covariate was identified and all assumptions were met.

From Table 5-2 it can be seen that independent *t*-tests showed no significant differences between the two groups. When age was used as a covariate in the analysis of HF and RMSSD the *P*-value moved closer to, but did not obtain statistical significance at the $P < 0.05$ level.

The final analysis of differences (Table 5-3) was carried out using non-parametric analysis (Mann-Whitney *U*-test) when data were close to or non-normally distributed or where heterogeneity of variances was shown between groups. This analysis also showed no significant differences between the values of HRV measures for the two groups.

Table 5-3. Non-parametric analysis of selected HRV measures.

HRV Measure	Reason for non-parametric analysis.	LO	HI	Mann-Whitney <i>U</i> -test.
TP (ms)	Close to non-normal distribution	3079.0 ± 2707.4	2568.3 ± 1869.2	Z = -0.17, P = 0.86
LF (ms)	Non-normal distribution	1343.5 ± 1914.9	2048.5 ± 2054.9	Z = -0.70, P = 0.94
HF (ms)	Non-normal distribution	411.3 ± 410.3	485.4 ± 614.6	Z = -0.84, P = 0.38
RMSSD (ms)	Heterogeneous variances	48.8 ± 31.5	48.5 ± 21.0	Z = -0.34, P = 0.73

Non-parametric analysis of selected HRV measures. Where non-normalcy of distribution is the reason for this analysis measures of central tendency are median ± interquartile range, all other values given are mean ± SD.

5.4. Discussion.

The aims of the present study were to examine differences in HRV measures between two groups of healthy subjects allocated on the basis of fitness and to examine the role of statistical analysis in explaining the heterogeneous nature of existing data from similar study designs.

By dividing the subject group according to $\dot{V}O_{2\ peak}$ attained on a maximal treadmill test, it was possible to compare subjects with high and low levels of fitness. The $\dot{V}O_2$ of the HI group was significantly greater than that of the low (29.1 ± 3.6 vs. 43.9 ± 3.2 ml·kg⁻¹·min⁻¹, $P = 0.001$). The magnitude of this difference is smaller than that reported in previous studies (Gallagher *et al.* 1992; Goldsmith *et al.* 1992; Davy *et al.* 1996). This is probably due to the sampling method used in the present study. Whereas previously subjects have been recruited purposely to enter either a fit or unfit group, here subjects were recruited as an opportunity sample and allocated to groups based on $\dot{V}O_{2\ peak}$ from maximal treadmill test performance. The HI subject group were also significantly younger than the LO group ($P = 0.001$) but they did not differ significantly on any other physical characteristic.

Analysis of HRV measures between the LO and HI groups found no significant differences. The results suggest that, in a normal healthy population, there are no significant differences in HRV measures between fit and unfit subjects.

Resting bradycardia has been observed in active, or fit subjects. In an attempt to explain the underlying causes of this bradycardia, differences in HRV markers of vagal tone have been measured in fit and unfit groups. Where cross-sectional comparisons of HRV measures have been made between athletes and sedentary controls, data suggest significant differences between groups on all HRV measures when 24-hour monitoring is used (Goldsmith *et al.* 1992; Jensen-

Urstad *et al.* 1997). Although not in unanimous agreement, short-term ECG measurements have produced some similar results.

Puig *et al.* (1993) found significantly greater values for time domain measures in a group of mixed (aerobically and anaerobically trained) athletes when compared to controls. Dixon *et al.* (1992) and Sacknoff *et al.* (1994) found increased vagal tone (HF) and reduced mixed vagal/sympathetic tone (LF) in endurance-trained athletes when compared to controls. Jannssen *et al.* (1993) found similar results using pNN50% as a marker of vagal tone and Shin *et al.* (1997) found no difference in LF but a significantly increased values for HF. This was taken as evidence for an increased vagal tone in athletes.

A number of studies show mixed results concerning HRV markers of vagal and sympathetic tone in athletes and controls. Aubert *et al.* (2001) found greater time domain measures of HRV in aerobically trained athletes than controls. Although they also found greater overall spectral power in this group, this was due to increased power in the LF band. Such findings demonstrate that differences between athletes and controls are not as homogenous as would perhaps be inferred if increased vagal tone is responsible for the bradycardia observed in athletes. Macor *et al.* (1996) found a greater percentage of HF power (HF%) in cyclists compared to controls but this was mainly due to greater LF power in the control group, as HF in raw units was similar between groups. Similarly, Shin *et al.* (1997) found a difference in HFnu but failed to provide raw units making confirmation of the reason for this difference difficult.

Results from short-term analysis therefore provide less than unanimous support for the notion that training induced bradycardia is due to increased vagal tone. More importantly they do not fully support the notion that chronic exercise training increases vagally-mediated measures of HRV.

When comparisons between controls and recreational athletes or fit and unfit subjects are made, the results remain equivocal. Some studies have shown very

large differences between active and sedentary subjects. Davy *et al.* (1997) found significantly higher TP, LF, HF and SDNN in active postmenopausal women compared to controls. The active groups were selected on the basis of habitual physical activity but the difference in fitness levels was very large. The fit group had almost double the $\dot{V}O_{2\max}$ of the unfit group (50.3 ± 2.5 vs. 26.7 ± 0.8 ml·kg⁻¹·min⁻¹). In the present study, the raw $\dot{V}O_2$ values were less distinct between groups to those above (44.1 ± 2.3 vs. 26.4 ± 3.3 ml·kg⁻¹·min⁻¹) which may go some way to explain why in the present study, as in the majority of other studies using this type of group allocation, reported results are negative.

In agreement with data from the present study, Gallagher *et al.* (1992) found no difference in LF:HF ratio between fit and unfit groups based on $\dot{V}O_{2\max}$ test scores. This was despite a difference between the groups of more than 40 ml·kg⁻¹·min⁻¹. Melanson (2000) allocated men to low, medium and high activity groups based habitual activity questionnaire results. He found no statistically significant differences between any of the groups although in the high activity group TP and HF were marginally higher than the low group ($P = 0.07$, $P = 0.06$ respectively). Migliaro *et al.* (2001) classified subjects as being either active or sedentary and verified groups allocation based on performance on an exercise stress test. Again, no significant differences in time or frequency domain measures were found.

The findings of the present study concur with previous data (Gallagher *et al.* 1992; Melanson 2000; Migliaro *et al.* 2001). There were no significant differences in either time or frequency domain variables between the groups despite a difference in $\dot{V}O_{2\max}$ of 14.8 ml·kg⁻¹·min⁻¹. The above studies used a variety of group allocation criteria and varied methods by which to analyse group differences. For instance, Gallagher *et al.* provide no statistic to describe the spread of their data. However, other measures they report such as RSA show heterogeneous variances it is therefore likely that the data used in the ANOVA

performed on their data may have violated the assumptions underlying the use of this test.

5.4.1. *Impact of statistical methods.*

Melanson (2000) used natural logarithms to allow the application of parametric analysis to his data, whereas Migliario *et al.* (2001) used a less powerful, non-parametric test to analyse their data. To assess the impact of these differences in data treatment data in the present study were carefully checked to ensure the assumptions underlying the statistical test used were not violated. Where appropriate, transformations were made data were again checked to ensure the success of this action. Additionally, non-parametric analyses were also used for comparability with previous data.

Following the application of all the above data treatments, still, no significant differences were found between the groups in the present study. This leads to the conclusion that differences in HRV measures are not significant between fit and unfit individuals.

The literature concerning HRV in the general population is in agreement that ageing is associated with reduced HRV (Coumel *et al.* 1994; Reardon and Malik 1996; Stein *et al.* 1997; Barnett *et al.* 1999; Kuo *et al.* 1999; Kuch *et al.* 2001; Parati *et al.* 2001). There is also evidence that it may be ageing *per se* and not associated reductions in physical activity which is responsible of this decline (Fukusaki *et al.* 2000). Although previous studies have used matched age groups, in the present study this was not done. Where age was found to be related to HRV measures it was added as covariate to control for its effects. This analysis led to the same conclusions being drawn as no differences were evident.

5.4.2. *Application of findings.*

The findings of the present study may, at first, seem to add little to the already existing knowledge base provided by previous data. However, studying two groups of healthy subjects divided in this manner provides insight into ambivalent findings from previous cross-sectional and longitudinal studies.

It seems that the differences in data treatment and statistical analysis are not the reasons for the equivocal nature in the existing literature. The reason for the differences in findings seems more likely to be due to the subject group studied. The majority of studies where large differences in HRV measures have been shown involve groups which differ greatly from each other in terms of fitness (Gallagher *et al.* 1992; Goldsmith *et al.* 1992). However, where the values of, for example, $\dot{V}O_{2\max}$ are less disparate between groups (Macor *et al.* 1996) no significant differences in HRV are reported.

Although the study of cross-sectional differences between disparate groups has several methodological advantages the applicability of findings to 'real world' situations is limited. Changes in $\dot{V}O_{2\max}$ due to training interventions are genuinely modest compared to cross sectional differences observed between groups. Where longitudinal changes in HRV have been studied, increases in $\dot{V}O_{2\max}$ due to training have varied, as have reported changes in HRV measures. Davy and Seals (1997) found no increase in $\dot{V}O_{2\max}$ or HRV measures following training in older women. Increases in $\dot{V}O_{2\max}$ of 4.3 ml·kg⁻¹·min⁻¹ (Boutcher and Stein 1995) 1.3 ml·kg⁻¹·min⁻¹ (Uusitalo *et al.* 2002) 1 and 4 ml·kg⁻¹·min⁻¹ (Loimaala *et al.* 2000) were all found to occur independently of significant changes in HRV. Similarly, two groups of older women who demonstrated training induced changes in $\dot{V}O_{2\max}$ of 3.4 ml·kg⁻¹·min⁻¹ and 4.7 ml·kg⁻¹·min⁻¹ showed no significant alteration in HRV (Perini *et al.* 2002).

al-Ani *et al.* (1996) reported changes in certain HRV measures following a mean change in $\dot{V}O_{2\max}$ of 6.0 ml·kg⁻¹·min⁻¹. Changes in HRV measures have also been

observed accompanying very large (16.5%) changes (Melanson and Freedson 2001) and very small (0.3%) changes (Portier *et al.* 2001) in $\dot{V}O_{2\max}$.

In common with the cross-sectional data, a number of the above studies have certain shortcomings in data treatment and analysis. Although the findings of the present study may suggest this may not be the reason for any lack of statistically significant differences being detected the importance of correct data treatment should still be stressed.

A reason for the equivocal nature of longitudinal findings may be due to the nature of measurement. HRV is a dynamic measurement and therefore subject to variation over short and long time periods. Numerous studies have reported poor reliability coefficients for HRV measurements, particularly short-term measures. This lack of reliability coupled with the high intersubject variation displayed by mean HRV measures reduces overall effect size (d) of any intervention. The simplest way to counteract this is to increase sample size, yet the majority of the existing studies have relatively small sample populations. If small sample sizes are used some remark as to the power of the study should be made. This may aid the reader in assessing the meaningfulness of the data reported.

None of the studies cited here give any evidence of power calculations in their methodologies including the present work. In this incidence it was because the data collected here were done so in an attempt to elucidate reasons for previous equivocal findings. Interestingly the power calculations made for the present study based on the work of Shin *et al.* (1996) for LFnu and HFnu yielded necessary sample sizes of $n = 22$ and $n = 6$ respectively. As an example of the variability in HRV measurements obtained in studies retrospective analysis revealed the power of the present study for HF was only $\beta = 0.1$. This illustrates that although power calculations should be carried they are not guaranteed to counteract methodological shortcomings.

Studies in this area, including the present one are, however, not without credit. From the current body of literature, meta-analysis be able to answer more fully, a

number of the research questions posed. This is again, not without problem. The existing data is disparate in nature concerning population age, sex and fitness levels. Cross-sectional data are based on comparisons of many different groups using numerous criteria. Longitudinal data contain many different exercise interventions in terms of type, duration, frequency and intensity.

To answer questions concerning the role of the autonomic nervous system in resting bradycardia or whether exercise interventions can alter HRV measures, some type of meta-regression may also be useful. This will then not only ascertain answers to the questions themselves but may also guide further empirical research and treatment as to the necessary nature of any intervention aimed at, for example increasing certain HRV measures through exercise.

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CHAPTER 6. PATTERNS IN TIME AND FREQUENCY DOMAIN MEASURES OF HEART RATE VARIABILITY IN RESPONSE TO EXERCISE: THE EFFECTS OF BASELINE HRV CHARACTERISTICS IN RESPONSE TO EXERCISE.

Abstract.

The primary aim of the present study was to observe changes in time and frequency domain measures of HRV derived from short-term recordings during three steady state exercise conditions. This was to verify the patterns of changes observed in previous studies using similar methodologies. A second aim of this study was to assess the effects of resting HRV characteristics on the response to exercise.

Twenty nine university staff and students (20 males, median age 39 range 19 - 63) and nine females median age 35 (range 19 - 56) were included in the study. Each subject underwent incremental treadmill testing to $\dot{V}O_2$ peak. On a separate occasion, subjects then underwent sequential, 5-min HRV recordings during supine rest, standing exercise at 25%, 50% and 75% $\dot{V}O_2$ peak. Effects of change in position and exercise were assessed using repeated measures ANOVA with *post hoc* repeated measures t-tests.

All time domain and absolute frequency domain HRV measures decreased significantly from supine to standing and through the three exercise intensities ($P < 0.001$). Normalised spectral power changed significantly between the conditions ($P < 0.05$) but did not behave in a uniform manner. When subjects were divided according to levels of sympathovagal balance at rest (using the low to high frequency power ratio LF:HF) there were significant interactions between groups ($P < 0.005$)

Results of this study agree with previous published data suggesting that recommended resting measures of HRV cannot suitably describe the changes in autonomic balance which occur during exercise, particularly at higher intensities.

Between-subject differences in sympathovagal balance may go some way to explaining the heterogeneity of findings in previously published studies.

6.1. Introduction.

One of the most profound effects of rhythmic exercise on the body is the increase in heart rate (HR). This increase is known to be a combination of at least three different mechanisms: the Frank-Starling law of the heart, humoral factors and the autonomic nervous system (ANS).

The behaviour of the autonomic nervous system has been well documented through the use of invasive measures of the sympathetic and parasympathetic branches of the ANS. This has led to the accepted theory that initial exercise induced tachycardia occurs due to parasympathetic withdrawal but that continued increases in HR at higher exercise intensities are brought about via sympathetic activation (Robinson *et al.* 1966; Maciel *et al.* 1986).

Since the observation of the rhythmic oscillations in RR interval was proposed as a possible non-invasive measure to assess the functioning of the short-term cardiovascular control systems (Akselrod *et al.* 1981) heart rate variability (HRV) has been frequently used as a marker of autonomic modulation of HR. Although most commonly used during resting conditions, short-term measures of HRV have been used to assess autonomic response to a number of stimuli including both physical and mental stressors (Pagani *et al.* 1995).

There are well-documented associations between HRV and autonomic function at rest. The study of HRV during exercise has yielded results inconsistent with accepted theory (Casadei *et al.* 1995) and concurrent, invasive markers of autonomic activity (Wallin *et al.* 1992; Breuer *et al.* 1993; Kingwell *et al.* 1994).

One consistent finding during exercise is a reduction in the overall variability in HR (Levy *et al.* 1998). This leads to reduced spectral power in all frequency bands (Perini *et al.* 1990; Perini *et al.* 2000; Perini *et al.* 2002). Findings concerning the remaining measures derived from RR interval variation are

inconsistent. This may in part be in part due to the varied methodologies reported in the literature.

Such variations include:

- The type of exercise undertaken
- The intensity of exercise
- The use of relative or absolute exercise intensities
- The use of single steady state, incremental or ramped exercise protocols
- The age, gender and physiological characteristics of the study population
- The length of the RR interval recording
- The mathematical methods used to transform RR intervals into the frequency domain

The aim of the present study was firstly, to observe changes in time and frequency domain measures of HRV derived from short-term recordings during three steady state exercise conditions. This was done to verify the patterns of changes observed in previous studies using similar methodologies. A second aim of this study was to assess the effects of resting HRV characteristics on the response to exercise.

6.2. Methods.

6.2.1. Subjects

Twenty nine university staff and students (20 males median age 39 range 19 - 63) and nine females median age 35 (range 19 - 56) were included in the study. All subjects were healthy, defined as being free from illness at the time of testing. None were known to be taking any medication or have any cardiovascular problems that may have influenced the tests carried out. All subjects volunteered to participate in the study. Written, informed consent was given separately for each part of the study: resting HRV measures, exercise testing and blood lactate testing. Subjects were, therefore, able to participate in the study without

consenting to blood sampling if they so chose. Complete blood lactate analysis was only completed on 20 subjects. One subject was excluded from the HRV analysis due to technical failure. Full HRV data during exercise was not collected on a large proportion of the subjects. This will be discussed further in the results and limitations sections.

6.2.2. Equipment.

6.2.2.1. Heart rate variability measurement.

HRV measures were made simultaneously using two instruments simultaneously; at rest and during exercise. Instrument one was a CardioPerfect ST 2001 HRV module (Cardio Control, Delft, The Netherlands). The system used a standard 12-lead ECG with a sampling frequency of 1000Hz. The sampling time for each HRV analysis was set at 5-min in agreement with current guidelines (Camm et al. 1996). The signal was digitised directly and the full ECG trace was shown in real time on a computer screen, this signal was simultaneously stored on the hard drive of a PC (Dell Computers, Texas, USA.) for post hoc analysis. Each sampling period was stored as a single patient ECG record. To analyse the variability in RR interval data an automated protocol within the HRV module software was used. The automated filtering system is adjustable, and a standardised configuration was used which treated the data as described below.

Editing of the raw RR interval data was carried out using an automatic-threshold-detection algorithm. The software rejected RR intervals which differed by more than 20% from the previous interval. This interval was then interpolated with an interval generated on the preceding interval data. The remaining analysis was based on the corrected data file of 'normal' RR intervals. The RR data were then passed through a Hanning type window to remove baseline trends. The RR interval time series was decomposed to the frequency domain via fast Fourier analysis. The resulting power spectrum was divided into the following spectral bands HF (0.15-0.40 Hz) LF (0.04-0.15 Hz)

and VLF (0.0033-0.04 Hz). In addition to this time domain analysis was also carried out to give the following values RMSSD, SDNN and RR interval.

The second HRV analysis instrument was a VariaCardio (TF5) HRV analysis system (Advanced Medical Diagnostics Group Ltd. Leeds, UK). This system used a purpose built chest-strap with two electrodes, placed either side of the subject's heart. The chest strap sampled at rate of 500 Hz. This signal was then sent to a receiver and digitised. The ECG trace and a graphical representation of RR interval were displayed in real time on the screen of a designated portable computer (IBM Systems USA). The sampling period for each measurement was set at 300 seconds or 300 beats whichever was longer. The instantaneous HR was derived from the identification of QRS complexes which was sampled at a higher rate of 1000 Hz. The RR interval and full ECG data were both stored on the hard disk of the designated portable computer prior for post hoc analysis.

Stored RR interval data were then edited automatically by the software. The manufacturers do not give details of the algorithm used to edit the data. To ensure effective editing all data were manually edited by a single researcher. This was done by visually analysing the raw ECG trace and the graphical representation of the RR interval data given. The researcher rejected intervals which appeared to represent potential artefacts, interference or noise. This filtered data set was then stored on the hard disk of the PC as a separate data file labelled filtered data. All further analysis was carried out on this filtered data set. The RR interval time series were decomposed to the frequency domain via fast Fourier analysis. The resulting power spectrum was divided into the following spectral bands HF (0.15-0.40 Hz) LF (0.04-0.15 Hz). Time domain analysis was also carried out to give the following values mean squared standard deviation (MSSD), SDNN and NN interval. The MSSD was then transformed to the RMSSD manually.

The reasons for the simultaneous use of two HRV systems were twofold. Firstly the dual analysis protocol was used to maximise the possibility of capturing the

maximal amount of data. This is because it is known, especially during exercise that changes in the signal-to-noise ratio may prohibit the spectral analysis of RR interval data (Gregoire et al. 1996). Secondly, although HRV analysis is now commonplace during exercise there are no data pertaining to the agreement between different analysis systems under such conditions.

6.2.2.2. Respiratory measurements.

The volume of expired air (V_E) and gas exchange measurements were made breath-by-breath, using a Medical Graphics CardiO₂ online breath-by-breath analysis system (Medical Graphics Corporation, St. Paul Minnesota, USA). Automated software from the same company (Breeze Suite) created a full set of nine-panel-plots (Wasserman et al. 1999) by which aerobic threshold could be detected. Blood lactate analysis was carried out using a YSI 1500 Sport lactate analyser (Analytical Technologies, Hanford House, UK).

6.2.3. Protocol

6.2.3.1. The Assessment $\dot{V}O_{2peak}$ and CPO_{peak} .

Subjects visited the laboratory on a total of three occasions to undertake different parts of the testing protocol. During visit one $\dot{V}O_{2peak}$ and CPO_{peak} were determined for each subject. Each subject completed a incremental exercise test (Bruce) on a motor-driven treadmill (Cardio Control, Delft, The Netherlands) to volitional exhaustion. This allowed an estimate of $\dot{V}O_{2peak}$ which was chosen in preference to true $\dot{V}O_{2max}$. $\dot{V}O_{2peak}$ has been found to be simple to measure, enduring standard of aerobic fitness (Mancini et al. 2000). It is less physically stressful than $\dot{V}O_{2max}$ for the subject and is therefore useful when using volunteers from clinical populations and the general population. Many subjects within these categories frequently have difficulty attaining true $\dot{V}O_{2max}$ as defined

by current guidelines (ACSM 2000). During the test \dot{V}_E and O_2 and CO_2 exchange were monitored continuously.

After at least 40 min rest the subjects performed a constant maximal workload exercise test on the treadmill. In this instance the researcher manually controlled the speed and gradient of the treadmill to elicit a $\dot{V}O_2$ close to (within 10%) the value achieved during the Bruce test. Cardiac output (CO) was then assessed using the CO_2 rebreathing technique (Defares 1960). Cardiac power output (CPO) was subsequently calculated using CO and blood pressure measured by a hand-held aneroid sphygmomanometer).

Two non-invasive methods are available to assess cardiac output the equilibrium method (Collier 1955) and the exponential method (Defares 1960). The exponential method was chosen as subjects experience significantly fewer adverse side effects compared with the equilibrium measure (Vanhees et al. 2000). There are data to support both the validity (Marks et al. 1985; Kuji et al. 1991) and reliability (Marks et al. 1985) of this method. Briefly, during the constant maximal workload test the subject's air supply was changed from room air to breathing from a bag containing 5% CO_2 and 14% O_2 for 10-20 s. The subject then rebreathed from this bag until a plateau in the increase in CO_2 concentration was observed. The increase in CO_2 and plotting of the CO_2 concentration during the manoeuvre was carried out by an automated protocol within the BreezeSuite software.

6.2.3.2. Calculations.

Cardiac power output was derived from the following equation:

$$CPO = CO \times MAP \times K \quad \text{Equation 6-1.}$$

Where MAP is mean arterial pressure, CO is cardiac output as derived from the indirect Fick principle via the rebreathing manoeuvre and K is the conversion factor (2.22×10^{-3}).

It was therefore first necessary to calculate mean arterial pressure by

$$\text{MAP} = (\text{SBP} + 2\text{DBP})/3 \quad \text{Equation 6-2.}$$

The estimation of cardiac output in this manner is based on the indirect Fick principle. The rebreathing manoeuvre gives an estimation of the mixed venous PCO_2 . The arterial CO_2 is estimated from the end tidal partial pressure of CO_2 . These two values are converted to concentrations automatically by dissociation tables within the MedGraphics software. These values are then substituted into the indirect Fick equation to give a measure of CO. Equation 6-1 is then used to produce CPO and heart rate obtained from successive RR intervals via the 12-lead ECG can also be used to calculate stroke volume.

6.2.3.3. Heart rate variability measurement.

During the second visit, each subject's resting HRV was analysed. In accordance with current guidelines for the capture of RR interval data for HRV analysis subjects attended the laboratory having refrained from eating or smoking for 2 hours. Subjects were also asked to refrain from alcohol or caffeine containing beverages and exercise on the day of the test, and exercise and heavy alcohol consumption on the evening prior to the test. Where subjects had failed to meet the requirements of the protocol an assessment was made on the potential impact of their behaviour on HRV measures and where necessary the test was rescheduled.

Disposable electrodes (Blue Sensor Medicotest, Olstykke, Denmark) were placed in the standard configuration for 12-lead ECG and the TF5 chest strap was placed laterally across the subject's heart in accordance with manufacturer's instructions. Subjects were then asked to lie on a bed in a quiet laboratory (18-22 °C). Subjects were asked to relax and the researchers monitored their HR visually until it became stable. Two simultaneous 5-min ECG recordings were then made. Two researchers were required to synchronously start both pieces of equipment. Although some auditory clues may have been available, the subject was purposely not informed of the start of the recording period. At the end of

the first recording the subject was asked to stand with feet shoulder width apart with hands placed on the back of a tall stool. Subjects were asked not to 'shuffle' or transfer their weight laterally during the data collection period. A second 5-min ECG recording was made when the subjects HR was deemed to be stable following the change in position.

6.2.4. *Measurement of HRV, blood lactate and CPO during incremental exercise.*

At the third visit to the laboratory, consenting subjects were first required to give a resting blood lactate sample. An arterialised capillary finger prick sample was taken from the index finger of the left hand and the whole blood was then transferred via micropipette to the YSI 1500 Sport Lactate analyser. All data were recorded from the analyser immediately and stored electronically for later analysis.

Subjects were then required to complete three stages of incremental exercise (25, 50 and 75% $\dot{V}O_{2peak}$). During this test ECG was continually monitored and breath-by-breath analysis of expired air was carried out using the techniques already described. The intensity of this exercise was determined from the $\dot{V}O_2$ at each level. When the level of predetermined $\dot{V}O_2$ was reached, a further 5-min ECG sample was taken. During this period, a blood pressure measurement was taken. At the end of 5-min a blood lactate measurement was made using the procedure described previously. At the end of each stage the subjects performed the rebreathing manoeuvre described earlier but with the addition of being instructed to breathe in time with a metronome eliciting a breathing rate of 0.15 Hz. This was done in accordance with the manufacturer's instruction to ensure the adequate rebreathing of the reference gas mixture from the bag.

6.2.5. *Data analysis.*

6.2.5.1. Data treatment and statistical analyses.

From the CardioPerfect system the following measurements were used: low frequency spectral power in raw (LF) and normalised units (LFnu), high frequency power in raw (HF) and normalised units (HFnu), the ratio of LF:HF, the root mean square of successive normal-to-normal intervals (RMSSD), the standard deviation of all normal-to-normal intervals (SDNN) and the mean RR interval (RR).

All data were tested for normality of distribution using a Shapiro-Wilk's test. Where distributions were found to be skewed logarithmic transformation (ln) was used to allow the use of parametric statistical analysis. Descriptive statistics were displayed as mean \pm SD unless otherwise stated and as raw or normalised units unless log transformation has been carried out (ln).

6.2.5.2. Heart rate variability during exercise.

To observe differences in these measurements over time, repeated-measures analysis of variance (rm-ANOVA) was applied. Data were first checked for the assumption of sphericity using Mauchly's test. Where the data displayed sphericity, significance at the given degrees of freedom for that comparison was accepted. Where the data were found to violate this assumption the given value of epsilon was used to adjusted (reduce) the degrees of freedom (and hence the value of P for any given value of F). As the assumption of sphericity is commonly violated in biological data, where no reasonable adjustment of df could be made the multivariate test value was taken. The calculation of this value does not require the assumption of sphericity. Where a significant main effect was found *post-hoc* analyses were carried using a repeated measures t -test out to identify points at which significant changes occurred without adjustment of

alpha. This was done despite the use of multiple comparisons being made to ensure findings were comparable to those of previous studies.

6.2.5.3. The effect of baseline characteristics on heart rate variability during exercise.

To study the effects of baseline characteristics on HRV responses to exercise the subject group was split into two groups. Those who showed vagal predominance (LF:HF>1) (VA) and those with sympathetic predominance (LF:HF<1) (SY) at baseline (supine). To assess the effect of gender on group allocation χ^2 analysis was used. To assess any interaction between condition and group a two-way mixed analysis of variance was used (mixed-ANOVA). *Post hoc* analysis between conditions was carried out using repeated measures *t*-test between groups. Independent *t*-tests were used for between group-comparisons where appropriate.

Although graphical representations of all data are provided, rm-ANOVA did not include data from the supine condition due to the large increase in between-condition variance this created. Therefore, all analyses were carried out on four conditions: standing 25%, 50% and 75% $\dot{V}O_{2\ peak}$.

In all cases, a value of $P \leq 0.05$ was taken as showing a significant difference. All analyses were carried out using SPSS version 11.0. SPSS Inc. (Chicago IL, USA).

6.3. Results.

6.3.1. *The effects of exercise on heart rate variability.*

The physiological characteristics of the subjects are given in table 6-1. The patterns of the RR interval (Figure 6-1) demonstrate a significant ($F = 3.6$, $P < 0.0005$) overall effect of exercise condition on RR interval. It should be noted that not all data are present in the 75% $\dot{V}O_{2\ peak}$ condition. This was due to low signal:noise ratio at this exercise intensity, which prevented the accurate detection of RR intervals.

Table 6-1. Descriptive statistics for all subjects (mean \pm SD)

	Males	Females
Age (years)*	36 (22 - 55)	40 (22 - 55)
Height (cm)	176.2 \pm 8.5	165.4 \pm 7.0
Mass (kg)	80.1 \pm 11.0	66.4 \pm 8.1
BMI	25.4 \pm 3.6	25.9 \pm 2.6
$\dot{V}O_{2\ peak}$ (ln)	3.2 \pm 1.2	2.8 \pm 1.1

*Median and range reported.

6.3.1.1. Time domain data.

Figure 6-2 shows the responses to exercise of the two time domain HRV measures used. There were significant effects for condition in both RMSSD ($F = 3.6$, $P < 0.05$) and SDNN ($F = 10.1$, $P < 0.0005$). *Post hoc* analyses revealed no change in RMSSD from standing to 25% or from 50 to 75%. Values for SDNN decreased significantly between all conditions except between 50 and 75%. Again, it should be noted that the data at 75% $\dot{V}O_{2\ peak}$ are representative of only $n = 19$ cases.

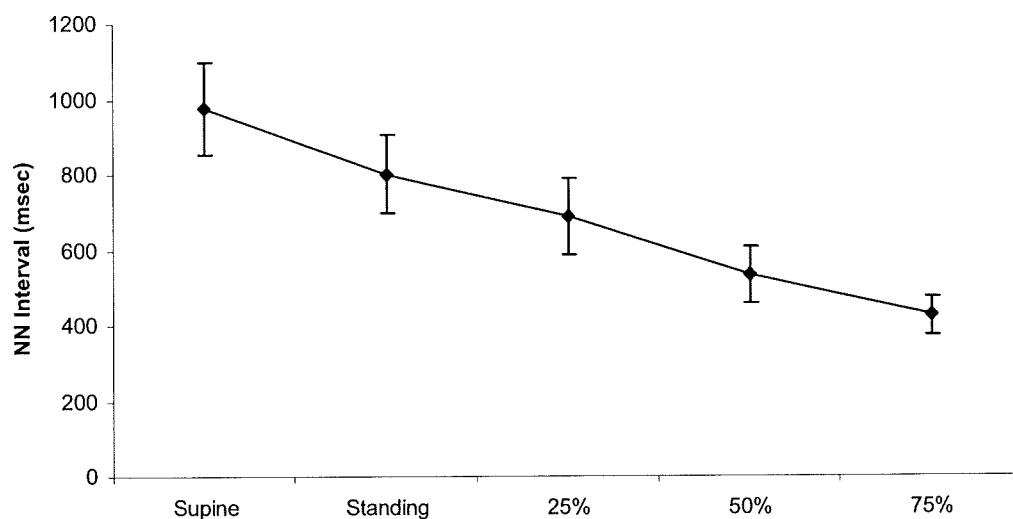


Figure 6-1. NN interval from supine rest to 75% $\dot{V}O_{2\text{peak}}$ in all subjects.

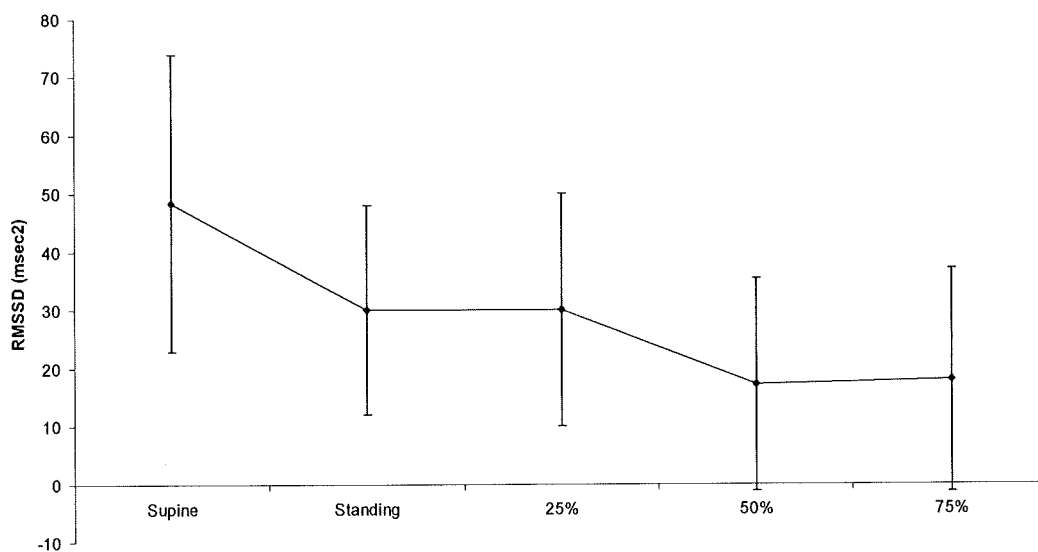


Figure 6-2. RMSSD from supine rest to 75% $\dot{V}O_{2\text{peak}}$ in all subjects.

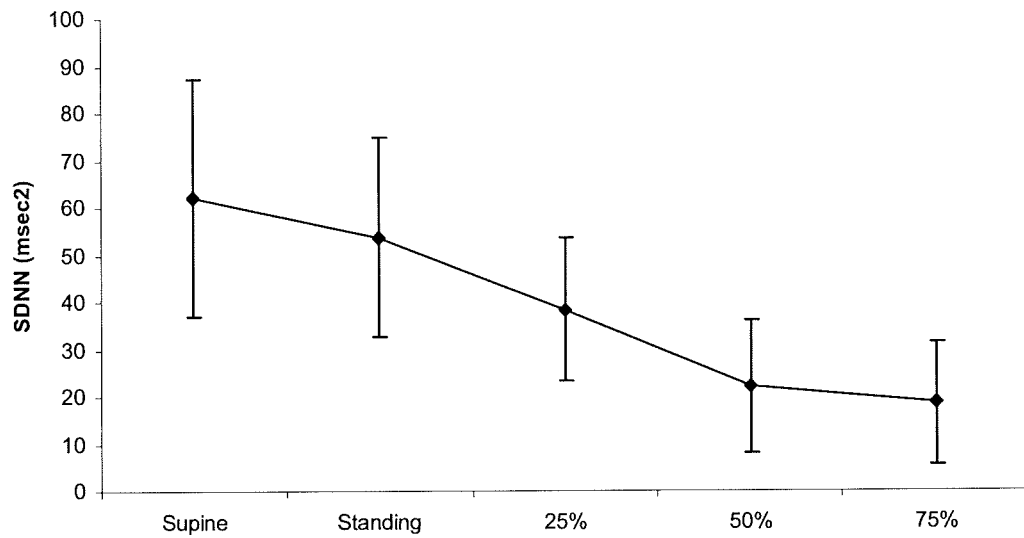


Figure 6-3. SDNN from supine rest to 75% $\dot{V}O_{2\ peak}$ in all subjects.

6.3.1.2. Frequency domain data.

Due to the skewed nature of the data, logarithmic transformations of the frequency domain data were carried out prior to statistical analysis. Figure 6-4 shows LF and HF in raw units (ms^2) in all conditions. There was a significant ($F = 61.1, P < 0.0005$) and HF ($F = 25.2, P < 0.0005$) from standing to 50% $\dot{V}O_{2\ peak}$.

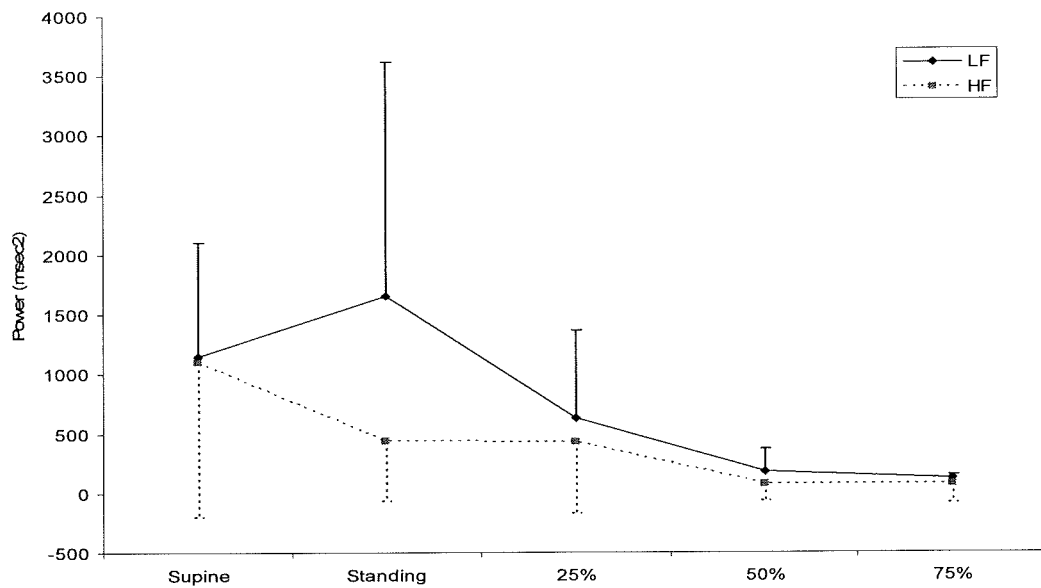


Figure 6-4. High and low frequency power (raw units) from supine rest to 75% $\dot{V}O_{2\text{ peak}}$ in all subjects.

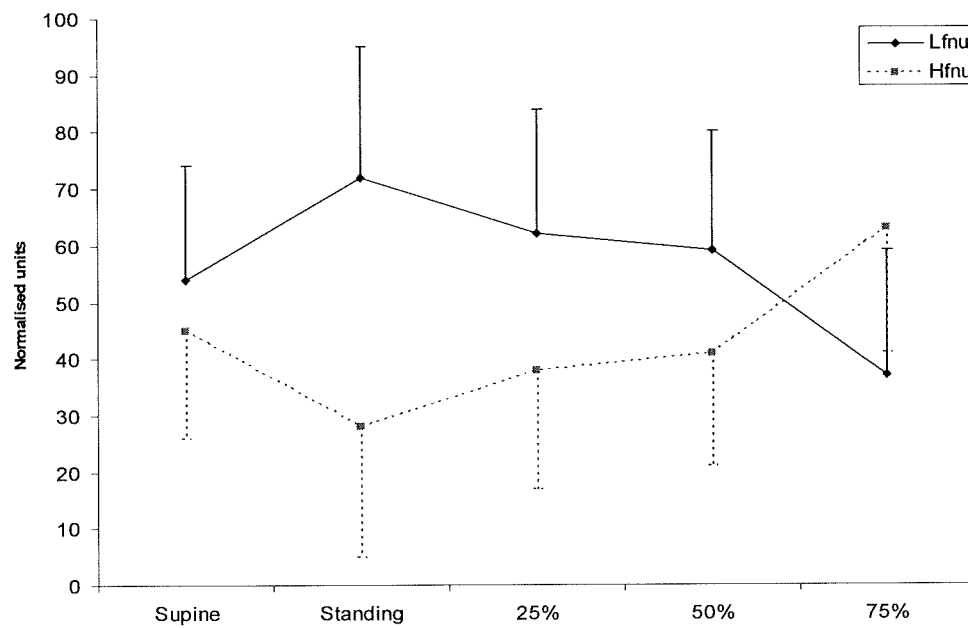


Figure 6-5. High and low frequency power (normalised units) from supine rest to 75% $\dot{V}O_{2\text{ peak}}$ in all subjects.

Power in both frequency bands was decreased as a function of exercise intensity (Figure 6-4). This was more pronounced in LF than HF. *Post hoc* analysis

(repeated measures t -test) showed LF decreased significantly ($P < 0.05$) between each condition except from 50-75% $\dot{V}O_{2\ peak}$. Power in the HF band remained unchanged from standing to 25% then only decreased significantly from 25 – 50% $\dot{V}O_{2\ peak}$.

When expressed in normalised units (Figure 6-5) rm-ANOVA showed no main effect of intensity ($F = 1.9, P > 0.05$). *Post hoc* analysis (repeated measures t -test) showed LF only decreased significantly from 50 to 75% $\dot{V}O_{2\ peak}$ ($t = 5.4, P < 0.0001$). There was also no significant overall effect of condition on HFnu ($F = 1.3, P > 0.05$). *Post hoc* analysis revealed HFnu remained unchanged from standing to 25% but increased significantly from 25 to 50% ($t = 5.4, P = 0.0001$) and from 50 to 75% ($t = 2.1, P < 0.05$).

6.3.2. *The effect of subject baseline characteristics of heart rate variability response to exercise.*

The data were split into groups dependent on sympathovagal balance at rest. Fourteen (3 females) subjects were placed in the VA group, the remainder made up the SY group (2 females). Analysis by χ^2 confirmed no effect of gender on group allocation.

6.3.2.1. Time domain measures.

During supine rest independent t -tests revealed no statistically significant differences between SY and VA groups in any time domain measures ($P > 0.05$). Analysis of RR interval data by mixed-ANOVA (Figure 6-6) revealed a significant main effect of condition ($F = 9.4, P < 0.0005$) but no significant interaction between the two groups ($F = 2.5, P > 0.05$). There was no main effect of condition on RMSSD ($F = 1.9, P > 0.05$) (Figure 6-7). Figure 6-8 shows the SDNN values for the SY and VA groups. There was a main effect of

condition ($F = 9.4, P < 0.005$) for SDNN but the group interaction observed failed to reach statistical significance ($F = 2.5, P > 0.05$).

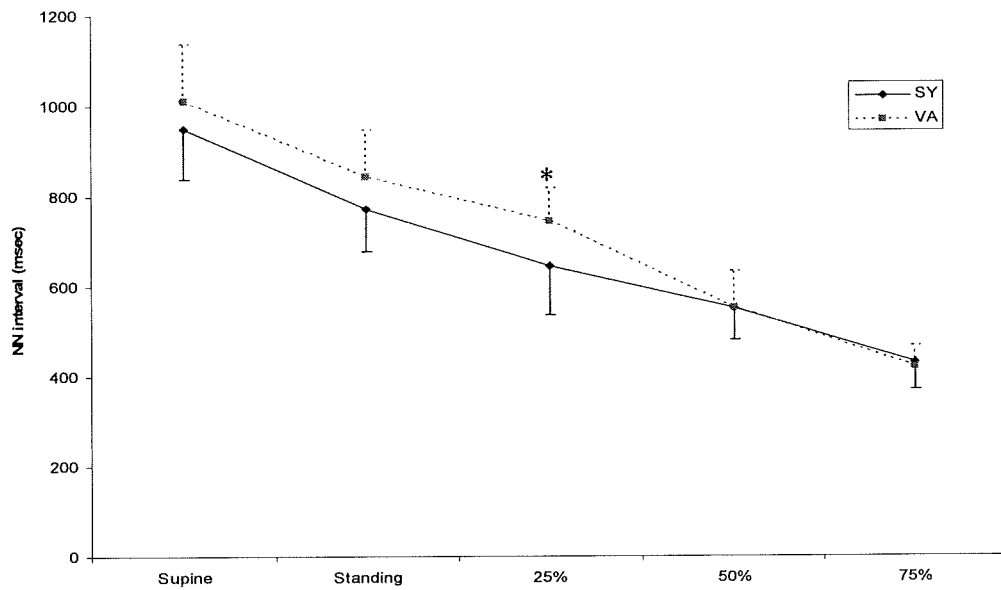


Figure 6-6. NN interval from supine rest to 75% $\dot{V}O_{2\text{ peak}}$ in subjects classified as vagally (VA) or sympathetically (SY) dominant at rest.

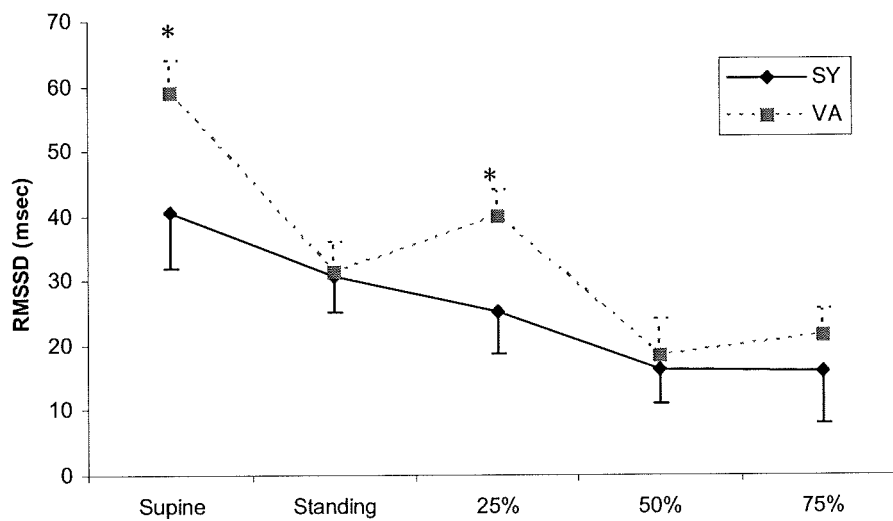


Figure 6-7. RMSSD from supine rest to 75% $\dot{V}O_{2\text{ peak}}$ in subjects classified as vagally (VA) or sympathetically (SY) dominant at rest.

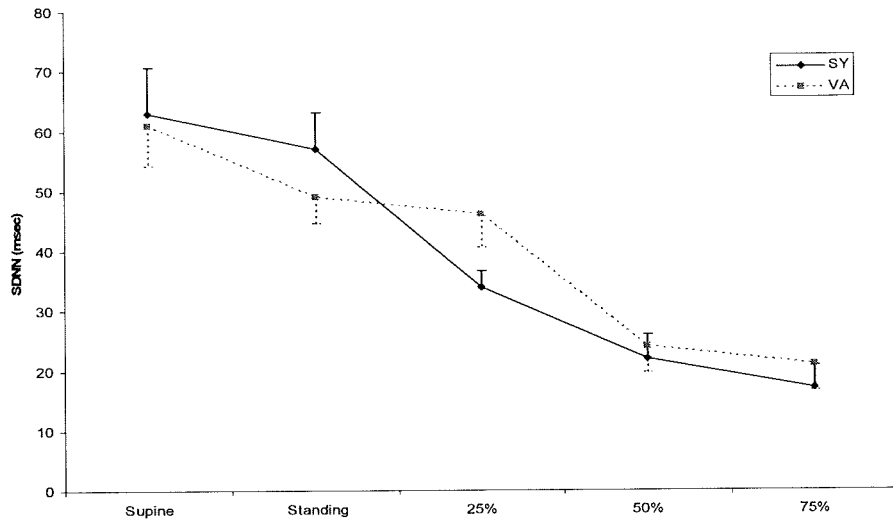


Figure 6-8. SDNN from supine rest to 75% $\dot{V}O_{2\text{ peak}}$ in subjects classified as vagally (VA) or sympathetically (SY) dominant at rest.

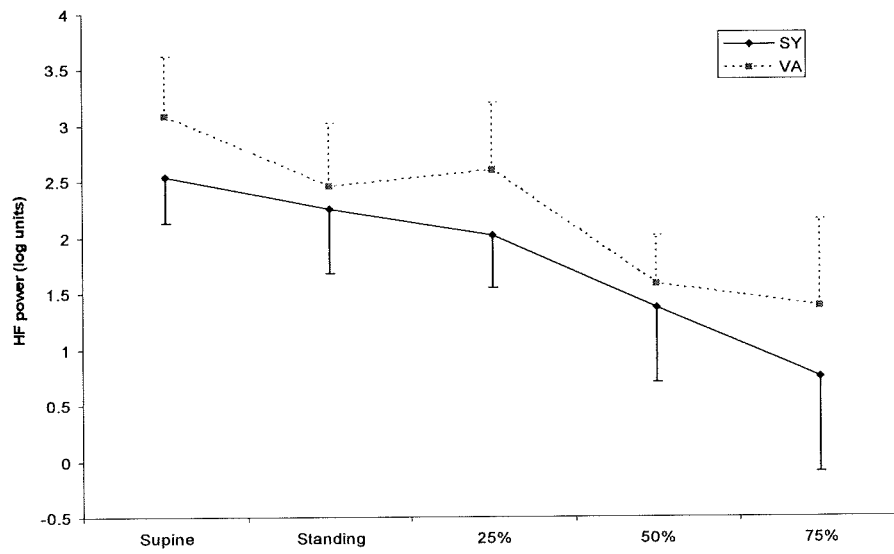


Figure 6-9. HF power from supine rest to 75% $\dot{V}O_{2\text{ peak}}$ in subjects classified as vagally (VA) or sympathetically (SY) dominant at rest.

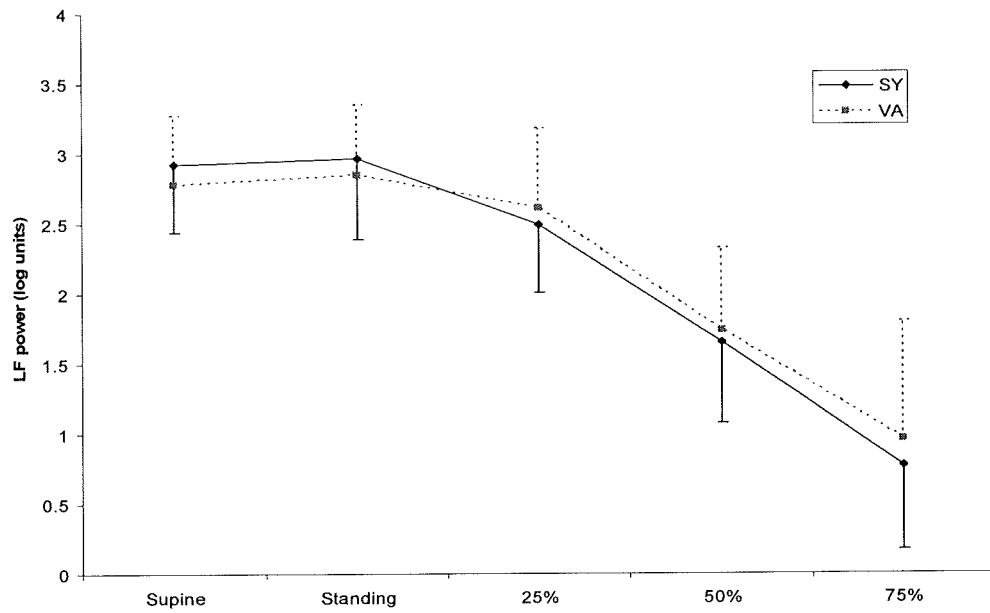


Figure 6-10. LF power from supine rest to 75% $\dot{V}O_{2\text{ peak}}$ in subjects classified as vagally (VA) or sympathetically (SY) dominant at rest.

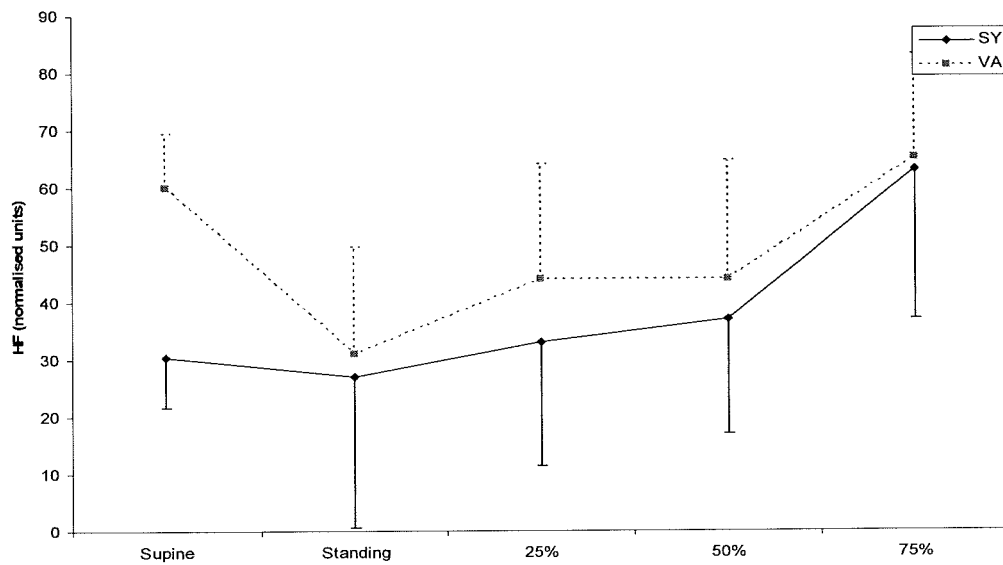


Figure 6-11. HFnu from supine rest to 75% $\dot{V}O_{2\text{ peak}}$ in subjects classified as vagally (VA) or sympathetically (SY) dominant.

6.3.2.2. Frequency domain measures.

Power in the HF band (log units) during supine rest was significantly higher ($t = 2.8, P < 0.05$) in the VA group (Figure 6-9). During standing and exercise, rm-ANOVA showed a significant main effect of condition ($F = 13.6, P < 0.005$) and a significant interaction between groups ($F = 6.4, P < 0.005$). Independent t -tests showed HF in the VA group slightly was higher when standing ($P > 0.05$) and significantly higher ($t = 3.8, P < 0.05$) at $25\% \dot{V}O_{2\ peak}$. It remained elevated at 50% although not significantly. This trend continued in the $75\% \dot{V}O_{2\ peak}$ condition where the difference remained, statistically significant ($P < 0.05$)

Analysis of LF power (log units) in the supine condition by independent t -test, revealed no difference between groups ($t = 0.76, P > 0.05$). Analysis of LF by rm-ANOVA (Figure 6-10) revealed a significant main effect of condition ($F = 46.0, P < 0.05$) but no significant interaction between groups ($F = 0.86, P > 0.05$). *Post hoc* analysis revealed no significant differences between groups in any condition.

There was a significant main effect for HFnu and LFnu ($F = 16.6, P < 0.05$) but no interaction between groups ($F = 0.25, P > 0.05$). *Post hoc* analysis showed no differences between groups in any condition (Figures 6-11 and 6-12).

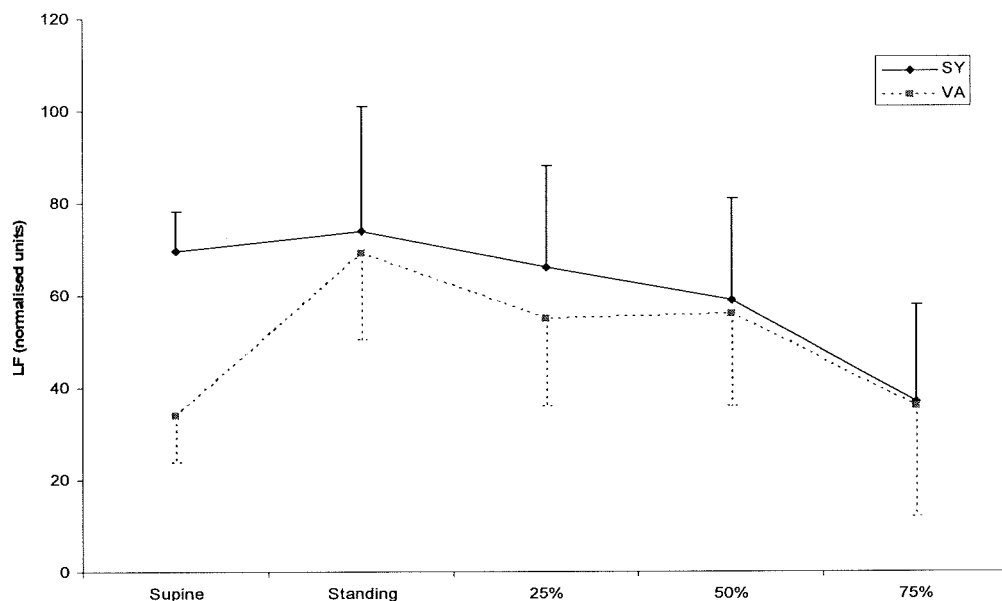


Figure 6-12 LFnu from supine rest to 75% $\dot{V}O_{2\text{ peak}}$ in subjects classified as vagally (VA) or sympathetically (SY) dominant.

6.4. Discussion.

6.4.1. Heart rate variability during exercise.

Studies concerning the effects of exercise on HRV measurements have used a variety of methodologies. Some have used only a single exercise intensity (Rimoldi *et al.* 1990; Kamath *et al.* 1991). Where multiple intensities have been used, HRV measurements have been made using a variety of exercise protocols including the following:

1. Ramping protocols with continuous ECG recording (Tulppo *et al.* 1996; Tulppo *et al.* 1998).
2. Three minute incremental stages using relative workloads with a 256 beat ECG recording period (Arai *et al.* 1989).

3. Five minute incremental stages using relative workloads with a 256-point beat ECG recording period. (Perini *et al.* 1990; Perini *et al.* 2000; Perini *et al.* 2002)
4. Discontinuous stages at workloads which elicit a given heart rate using a 256 s ECG recording period (Breuer *et al.* 1993).
5. Five minute stages at absolute workloads with a 256 beat ECG recording made in the last 2-3 min (Casadei *et al.* 1995).

Few studies give information concerning the stability of the tachograms analysed and it seems that none of the above studies have recorded steady state ECGs for a full 5-min at relative workloads. Therefore, although the present study is comparable to several previous studies (Arai *et al.* 1989; Perini *et al.* 1990; Casadei *et al.* 1995; Perini *et al.* 2000; Perini *et al.* 2002) it remains methodologically unique.

The main findings of the present study were threefold. Firstly, compared to resting values there was a decrease in the variation of RR interval. This led to a reduction in spectral power of the time series recorded over five minutes of steady state exercise. Secondly, at rest, HFnu and LFnu are commonly considered markers of vagal and mixed vagal/sympathetic activity respectively. During exercise, neither measure behaved as would be expected if this were still true. Thirdly, when subjects were divided by vagal or sympathetic predominance at rest significant differences in the changes in LF and HF were found during exercise. These findings will be discussed separately in detail in the following sections.

6.4.1.1. Time domain measures of HRV during exercise.

An almost linear reduction in total HRV as measured by SDNN was evident with increasing exercise intensity. It is known that SDNN correlates with pharmacologically derived indices of vagal activity (Uusitalo *et al.* 1996). These findings therefore fit with the accepted theory regarding vagal withdrawal during

exercise. Similarly, RMSSD also correlates with vagal activity. No reduction was found between standing to 25% $\dot{V}O_{2\ peak}$. However, RMSSD decreased between 25 and 50% $\dot{V}O_{2\ peak}$. Although some reduction was evident neither RMSSD nor SDNN declined significantly from 50 – 75% $\dot{V}O_{2\ peak}$. This may indicate that complete vagal withdrawal had occurred by 50% $\dot{V}O_{2\ peak}$. These findings concur with those previous studies which have demonstrated reduced values for time domain measures with increased exercise intensity (Perini *et al.* 1990; Tulppo *et al.* 1996; Levy *et al.* 1998; Tulppo *et al.* 1998; Perini *et al.* 2000; Perini *et al.* 2002).

6.4.1.2. Frequency domain measures.

When the time series of RR intervals is transformed into the frequency domain there was reduced spectral power in the LF and HF bands. This finding is also in agreement with previous general spectral analysis (GSA) studies which have used fast Fourier transformation (FFT) (Breuer *et al.* 1993; Gregoire *et al.* 1996; Myslivecek *et al.* 2002) and those which have used autoregressive modelling (Arai *et al.* 1989; Bernardi *et al.* 1990; Perini *et al.* 1990; Kamath *et al.* 1991; Casadei *et al.* 1995; Tulppo *et al.* 1996; Tulppo *et al.* 1998; Perini *et al.* 2000; Perini *et al.* 2002).

From supine to standing there was a significant ($P < 0.05$) increase in LFnu confirming this measure's known association with sympathetic activation brought on by orthostatic stress. This finding agrees with others that have either compared supine and standing values (Pagani *et al.* 1986; Byrne *et al.* 1996) and recorded HRV during passive tilt (Furlan 1987; Furlan *et al.* 2000) or active standing (Jauregui-Renaud *et al.* 2001). Standing was associated with reduced HFnu. This also agrees with previous data and has been interpreted as being representative of vagal withdrawal. Given the increase in LFnu between supine and standing conditions, it would be expected that LFnu and HFnu would continue to increase and decrease respectively during exercise. This seems

logical as exercise is a condition known to augment sympathetic outflow. This was not found to be the case. At 25% $\dot{V}O_{2\ peak}$ HFnu was found to increase and a reciprocal decrease in LFnu was observed. Although unexpected, similar findings have been reported previously in young (Warren *et al.* 1997) and elderly (Perini *et al.* 2002) subjects. More commonly however, at lower exercise intensities LFnu and HFnu have been found to increase and decrease respectively (Bernardi *et al.* 1990; Rimoldi *et al.* 1992; Casadei *et al.* 1995). This is commonly interpreted as demonstrating parasympathetic withdrawal and/or sympathetic activation in line with current theory regarding the origins of exercise tachycardia. In the study of Perini *et al.* (2002) the changes in LFnu and HFnu were interpreted as reduced sympathetic activation in their elderly subjects, Warren *et al.* (1997) cite methodological reasons for their findings. However, in the healthy, non-elderly population studied here, it would be expected that LFnu would increase.

One explanation for the conflicting results between this study and previous data may be due to methodological differences. In the present study LFnu in the standing condition may have been elevated for two reasons. For most subjects, HRV was measured in the supine and standing conditions on separate occasion to exercise measures. The resting measures were made on the first occasion which subjects had undergone HRV recording. In a small cohort it was necessary to record HRV prior to the onset of the exercise test. Both these conditions (an unfamiliar protocol and an impending exercise test) may have elevated psychological stress levels in subjects. This has previously been shown to elevate LFnu (Pagani *et al.* 1995). It is possible that as the subject commenced exercise they may have relaxed as their attention was drawn toward the task. A second explanation for the fall in LFnu may lie with the workloads used. Studies which have shown raised LFnu at the onset of exercise have typically used absolute workloads. In the present study, a relative workload of 25% $\dot{V}O_{2\ peak}$ was used as the initial stage. For all subjects this was very light exercise. In some cases such a low oxygen consumption was difficult to maintain during treadmill exercise.

The mean value for oxygen consumption all subjects throughout the first stage of exercise was actually 13% higher than the target value corresponding to of 25% of predetermined $\dot{V}O_{2\ peak}$.

At 50 and 75% $\dot{V}O_{2\ peak}$ LFnu was significantly reduced. This produced conversely, a significant rise in HFnu. These, and similar findings from previous studies (Casadei *et al.* 1995; Bernardi *et al.* 1996) would lead to the somewhat unlikely conclusion that sympathetic activity is decreased at higher intensity exercise. Even more unlikely, the rise in HFnu suggests increased vagal activity.

One reason for such findings may lie within the origins of the LF and HF fluctuations themselves. Although HF has known to be of vagal origin, the belief that LF represents mainly sympathetic outflow (Pagani *et al.* 1986; Malliani *et al.* 1991) has been challenged on numerous occasions (Eckberg 1997; Goldberger 1999). Pharmacological data suggest fluctuations in the LF band to be at least partially vagally mediated (Akselrod *et al.* 1981; Pomeranz *et al.* 1985). This may go some way to explaining the reduction in spectral power in both frequencies observed during exercise. This does not, however, explain the increase in the relative shift of power into the HF spectral band during exercise.

Pharmacological data have been shown to be able to eradicate the LF spectral peak under dual blockade (Pagani *et al.* 1986). Data from the denervated hearts of transplant patients (Bernardi *et al.* 1990) support the view that LF is purely autonomic in origin. Fluctuations in the HF band may still be observed under complete pharmacological blockade and in the denervated heart (Bernardi *et al.* 1990). Oscillations in the HF band may therefore be non-neural in nature. Animal data suggest alterations in intramural pressure (Blinks 1965) and myocardial stretch (Kohl *et al.* 1994) may both be able to causes synchronous depolarisation of cells near the SA node resulting in oscillation in nodal firing at frequencies within the HF band. In an attempt to quantify the input of these

mechanisms (Casadei *et al.* 1996) it was estimated that more than 40% of HF power may be of non-neural origin during moderate exercise.

During exercise there two factors are which may increase HFnu. Firstly, overall spectral power is reduced. This occurs in both spectral bands and is mainly due to changes in nervous activity. Secondly, as respiratory depth and frequency increases during exercise the non-neural power input to the spectrum, which contributes exclusively to the HF band, increases. The combination of reduced spectral power from neural input, combined with preserved HF power from non-neural origins artificially inflates HFnu and reciprocally decreases LFnu. Therefore, at higher exercise intensities the use of HFnu and LFnu and the LF:HF ratio may be misleading. Further to this, there are practical issues concerning data collection at high ($75\% \dot{V}O_{2\ peak}$) exercise intensities. In the present study only 14 full data sets could be recorded. In other records, a low signal:noise ratio meant in some cases more than 50% of the RR intervals analysed were interpolated.

6.4.2. *Differences in heart rate variability response to exercise in relation to subject baseline characteristics.*

Despite problems with the use of GSA during exercise, a number of studies have been carried out, successfully examining the role of ANS control during low intensity exercise. The remainder of this discussion will relate only to data collected in the supine, standing and $25\% \dot{V}O_{2\ peak}$ exercise condition.

As mentioned previously, in transition from rest (standing due to orthostatic stress) to exercise the data is somewhat equivocal regarding LFnu and HFnu. This may in part be due to subject characteristics.

Arai *et al.* (1989) found that when subjects exercised at an intensity which produced a HR of 120-140 BPM an increase in LF:HF ratio was evident.

Likewise, Bernardi *et al.* (1990) exercised subjects at 120 W and found an increase in LFnu. Kamath *et al.* (1991) found a small increase in LFnu when subjects cycled at 50% of maximal predicted power as did Breuer *et al.* (1995) when subjects cycled at a workload eliciting a HR of 100 BPM. Casadei *et al.* (1994) also found increased LFnu at the absolute workload of 110W.

Perini *et al.* (1990) used 21% $\dot{V}O_{2\max}$ as their lowest exercise intensity and found a decrease in LF:HF. Similarly, Warren *et al.* (1997) found a decrease in LF:HF when subjects cycled at 0 W. Using elderly subjects, Perini *et al.* (2000) found reduced LF at an exercise intensity which produced a $\dot{V}O_2$ of 10 ml·kg·min⁻¹. In a further study (Perini *et al.* 2002) this direction of change was reversed following an aerobic training programme, when subjects exercised at an intensity eliciting a $\dot{V}O_2$ of 0.3 L·min.

In the present study both LF and HF were reduced as a function of exercise intensity when the entire study population was analysed. When subjects were divided into SY and VA groups the reductions in the latter were much less pronounced from supine to standing and 25% $\dot{V}O_{2\text{peak}}$. This indicates a preservation of HF power during orthostatic stress and exercise in this group. It has previously been suggested that such a preservation of HF power may be cardioprotective in nature (Davy *et al.* 1996) as it may reduce the likelihood of arrhythmia. Animal data support this suggestion (Hull *et al.* 1994). Data from the present study are illustrative of the influence baseline HF and LF may have on changes in these measures during exercise. These data may also be helpful in explaining the diversity of findings related to changes in LF and HF during exercise.

The large decreases in spectral power observed in the LF and HF bands during exercise create difficulties in making inferences from exercise HRV data. Numerous researchers have reported findings in normalised units in an attempt to overcome this problem.

Findings from GSA concerning the behaviour of LFnu and HFnu during exercise have been disparate in nature. Given the respective vagal and sympathetic modulation of HFnu and LFnu assigned by some authors (Pagani *et al.* 1986; Malliani *et al.* 1991) a decrease in the former and increase in the latter would be expected during exercise. Such patterns of change have been shown previously (Yamamoto and Hughson 1991; Perini *et al.* 2000). However, the data are not homogenous, and decreases in LFnu (and an accompanying increase in HFnu) during the onset of exercise have also been reported (Kamath *et al.* 1991; Rimoldi *et al.* 1992). In older men and women, Perini *et al.* (2002) found an immediate decrease in LFnu (increased HFnu) at the onset of light exercise when measurements were made prior to exercise training. Following an effective (increased $\dot{V}O_{2\max}$) training period this pattern was reversed; subjects showed increased LFnu and increased HFnu. These changes were not accompanied by any changes in LFnu or HFnu at rest. In the present study there was interaction between SY and VA groups during exercise. These data support the findings of Perini *et al.* (2002) and suggest factors other than subject baseline characteristics may be responsible for the disparate nature of data from previous studies using these measures.

The findings of Perini *et al.* (2002) suggest changes in subject fitness level may be associated with alterations in the responses of LFnu and HFnu during exercise. Further investigation of the relationship between subject fitness or exercise performance and HRV exercise response may therefore be warranted.

6.5. Conclusions.

Findings from HRV studies during exercise are disparate in nature. This study demonstrates that the heterogeneous nature of findings may be in part, a result of differing subject baseline HRV characteristics. If GSA is to be used as a tool to investigate autonomic control during exercise, there are several methodological issues that need to be resolved particularly concerning measures taken from ECG

recordings at higher exercise intensities ($>50\% \dot{V}O_{2\max}$). Due to reduced spectral power at higher intensities it seems that GSA, in its present form, is most useful only during light exercise. The opposing behaviour of LFnu and HFnu to that which would be expected as respective sympathetic and vagal markers is problematic. Further work is required to elucidate causes for the differing behaviour of these measures reported during exercise.

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CHAPTER 7. THE DEVELOPMENT OF A MODIFIED, GENERAL SPECTRAL ANALYSIS HEART RATE VARIABILITY FRAMEWORK FOR USE DURING EXERCISE.

Abstract.

The aim of the present investigation was to assess whether valid indices of vagal and sympathetic activity could be generated through the manipulation of spectral HRV measures within the limits imposed by commercially available analysers.

Twenty nine university staff and students (20 males median age 39 range 19 - 63) and nine females median age 35 (range 19 - 56) were included in the study. Each subject underwent incremental treadmill testing to $\dot{V}O_2$ peak. On a separate occasion, subjects then underwent sequential, 5-min HRV recordings during supine rest, standing exercise at 25%, 50% and 75% $\dot{V}O_2$ peak. Vagal modulation was measured using HF power measured from 0.1- 0.5 Hz and sympathetic modulation was measured using the ratio of LF (0.01 – 0.1Hz)/TP (0.01-0.5 Hz). Effects of exercise on these measures were assessed using repeated measures ANOVA with *post hoc* repeated measures *t*-tests.

Results showed a significant decrease in HF from rest through all exercise intensities ($P < 0.0001$). *Post hoc* analyses showed that the largest changes in HF were between 25 and 50% $\dot{V}O_2$ peak. LF/TP was altered significantly across all conditions ($P < 0.001$). LF/TP was unchanged from rest to 25% $\dot{V}O_2$ peak but increased from 25% $\dot{V}O_2$ peak to 50 % $\dot{V}O_2$ peak. At 75% $\dot{V}O_2$ peak there was a small decrease in LF/TP.

The results of the study demonstrate that HF power measured from 0.1- 0.5 Hz provides a good index of vagal activity during exercise. The ratio of LF (0.01 – 0.1Hz)/TP (0.01-0.5 Hz) was put forward as an index of sympathetic activity. Although some of the behaviour of this measure during exercise suggest it may

be of some use as a marker of sympathetic activity the majority of the results suggest that this is at best, an equivocal marker. Additionally its use may be limited to lower ($<75\% \dot{V}O_{2\text{max}}$) exercise intensities. Low signal:noise ratio made recording and analysis of the ECG data at high (75% $\dot{V}O_2$ peak) exercise intensities difficult.

7.1. Introduction.

In a previous chapter the effects of exercise on HRV measures obtained by general spectral analysis (GSA) were examined. The findings were found to be in agreement with previous data. They showed an overall decrease in global measures of HRV in the time and frequency domains.

The behaviour of time domain measures (RMSSD, SDNN) from short-term HRV measurement fits reasonably well with classical theory regarding autonomic changes during exercise (Robinson *et al.* 1966; Frick *et al.* 1967; Sutton *et al.* 1967; Ekblom *et al.* 1973; Nyberg 1981). The behaviour of frequency domain measures during exercise, does not fit well with their assigned roles as makers of vagal (HF, HFnu), or sympathetic (LF, LFnu) tone, or of sympathovagal balance (LF:HF ratio). Although there are much data to offer support for frequency domain measures as non-invasive markers of autonomic control at rest, the behaviour of these variables during exercise is commonly found to be paradoxical to their behaviour at rest.

What follows is a brief discussion of each frequency domain variable recommended for use from short-term analysis by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (Camm *et al.* 1996). Each variable will be discussed in terms of:

- Its role as an autonomic marker at rest.
- Physiological and pharmacological data to support or refute this role.
- Its behaviour during exercise and the congruency between this and resting data.

7.1.1. High frequency power (HF).

This distinct peak in the frequency spectrum represents respiration-mediated regulation of HRV. It is reduced under conditions of orthostatic stress such as standing (Janssen *et al.* 1993) and tilt (Pagani *et al.* 1986; Montano *et al.* 1994; Vardas *et al.* 1994; Bootsma *et al.* 1996) compared to when it is measured in the supine position. It is totally abolished by atropine (Pomeranz *et al.* 1985; Rimoldi *et al.* 1990; Uusitalo *et al.* 1996) and glycopyrrolate (Warren *et al.* 1997) suggesting purely vagal modulation. However, direct, microneurographic evidence is more equivocal. Whereas a direct relationship between HFnu and vagal activity has been demonstrated (Pagani *et al.* 1997), more recently HF has been found to demonstrate no relationship with microneurographically measured vagal activity (Notarius *et al.* 1999).

Despite this, the majority of evidence suggests HF to be a good marker of vagal activity. Therefore, during exercise it would be expected that HF in raw units (ms^2) and in normalised units (HFnu) would decrease at lower exercise intensities indicating vagal withdrawal. At higher exercise intensities there should be no distinct HF peak.

The behaviour of HF follows such a pattern. The spectral power in the HF band (0.15 – 0.4 Hz) is dramatically reduced with increasing exercise intensity (Arai *et al.* 1989; Rimoldi *et al.* 1990; Tulppo *et al.* 1998), as is power in the entire, measured, spectral range (commonly 0.04 – 0.4 Hz). When HF is expressed in normalised units (HFnu), dramatic reductions in TP often mean HF represents a greater proportion of TP, therefore increasing the normalised value. This, paradoxically, may be interpreted as an increase in vagal activity during intense exercise (Casadei *et al.* 1995). At very high exercise intensities HF power may actually increase. There is evidence that the source of this increase may be non-neural (Casadei *et al.* 1995) and linked to mechanical stimuli due to increased breathing effort (Bernardi *et al.* 1990).

In summary, it seems that the behaviour of HF and HFnu at lower exercise intensities is congruent with its assigned role as a vagal marker at rest. However,

at moderate and high intensities it seems that paradoxical rises in this vagal marker may invalidate its use.

7.1.2. *Low frequency power (LF).*

The role of the LF peak in the power spectrum (0.04 -0.15 Hz) is much less clearly defined than that of HF. There is evidence to suggest LF is linked to baroreceptor mechanisms (Kamath and Fallen 1993) and Meyer waves (Furlan 1987). Contradictory evidence for a central origin of LF also exists (Cooley *et al.* 1998). Studies using orthostatic stress tests show shifts toward LF predominance due to tilt (Pagani *et al.* 1986; Malliani *et al.* 1991; Montano *et al.* 1994), although data are somewhat equivocal (Saul *et al.* 1990). Evidence of attenuated increases in LF power in response to tilt under β -adrenergic blockade suggest a sympathetic origin (Rimoldi *et al.* 1990), although the findings of published studies do not always concur (Hopf *et al.* 1995; Introna *et al.* 1995).

Data from cholinergic blockade also suggest a strong vagal influence on LF (Akselrod *et al.* 1981) particularly in the supine position (Pomeranz *et al.* 1985). Paradoxically, LF power under vagal blockade may be reduced (Vybiral *et al.* 1990) despite reported increases in RR interval (Raczkowska *et al.* 1983). This, and findings of increased LF under β -adrenergic blockade (Cacioppo *et al.* 1994; Jokkel *et al.* 1995) have led to the opinion that LF may be a poor marker of sympathetic activity (Appel *et al.* 1989; Houle and Billman 1999). Microneurographic evidence, paradoxically suggests both a link (Pagani *et al.* 1997) and direct dissociation (Notarius *et al.* 1999) between LF and direct measures of sympathetic activity. Simultaneous assessment of LF activity has also been found to be discordant with the gold standard of cardiac sympathetic activity; direct measurement of noradrenaline spillover (Kingwell *et al.* 1994).

It is clear from the contradictory nature of the evidence that to consider LF (or LFnu) as a purely sympathetic index would be both simplistic, and incorrect. A consensus viewpoint (Stein and Kleiger 1999) has been put forward that LF is at

best a complex and mixed (vagal and sympathetic) component of HRV. Additionally, LF may be a marker of sympathetic activity under certain conditions such as tilt.

Due to the equivocal nature of the data, it is difficult to predict how LF should act during exercise. Those proponents of LF and especially LFnu as markers of sympathetic predominance would expect an increase in both measures with increasing exercise intensities. If LF and LFnu are interpreted as mixed vagal-sympathetic markers, a decrease in LF but an increase in LFnu would be expected. Neither of these scenarios is consistently supported by empirical data.

When expressed in raw units, LF uniformly shows a decrease during exercise when compared to resting conditions (Arai *et al.* 1989; Rimoldi *et al.* 1990; Breuer *et al.* 1993). When normalised for the overall reduction in TP, LFnu has been reported to increase from rest to very light exercise (Perini *et al.* 1990; Casadei *et al.* 1995; Perini *et al.* 2000; Perini *et al.* 2002). However, during incremental exercise the proportion of TP represented by LF (LFnu) is most commonly observed to decrease as a function of exercise intensity (Perini *et al.* 1990; Kamath and Fallen 1993; Casadei *et al.* 1995; Perini *et al.* 2000; Perini *et al.* 2002). Maximal exercise is usually accompanied by a large decrease in LFnu as HF oscillations make up a much greater proportion of TP.

From the above evidence it can be stated that LF is at best an equivocal marker of sympathetic activation at rest. During exercise, the decreases in LF and LFnu observed directly contradict the known sympathetic activation that occurs at higher intensities. On this basis, LF and LFnu are not useful indices of cardiac sympathetic activation during exercise.

7.1.3. *Low frequency to high frequency ratio (LF:HF ratio).*

Proponents of the LF:HF ratio (Pagani *et al.* 1986) recommend its use as an index of sympathovagal balance. Forgoing the previous discussion of the origins

and nature of LF, LF:HF seems an intuitively inviting concept by which the balance between sympathetic and vagal actions on the SA node may be measured. Taking into account the shortcomings of both the numerator and denominator in this equation it is obvious that the LF:HF ratio has numerous problems associated with its use as a measure of sympathovagal balance.

Using upright tilt, it has been shown that LF:HF increases with the angle of tilt (Iwase *et al.* 1987; Montano *et al.* 1994; Mukai and Hayano 1995). However, both Montano *et al.* (1994) and Muakai *et al.* (1995) report the change in LF:HF to be due to decreased HF (indicating vagal withdrawal) as opposed to increases in LF (to indicate sympathetic activation). This illustrates an inherent weakness in the use of a ratio to describe sympathovagal balance. The use of a ratio means that mathematically, one component must always change in light of the activity of the other. Physiologically there is no evidence for any such behaviour of the vagal and sympathetic inputs to the SA node. The mathematical, linguistic and philosophical nature of LF:HF have been challenged (Eckberg 1997; Goldberger 1999). A full discussion of these points is beyond the scope of the present text, but empirical data suggesting further weaknesses of this ratio. Using direct measurement, some subjects have been found to decrease their sympathetic nerve traffic when in the upright position (Burke *et al.* 1977). Wallin *et al.* (1992) found tilt to be effective in increasing noradrenaline spillover from the myocardium, but found this increase was not accompanied by increased power in the LF band.

The majority of data from passive tilt do seem to support the use of LF:HF as a sympathovagal marker (Furlan 1987). However, under exercise conditions, the behaviour of the ratio does not conform to that which would be expected if it is indeed, representative of the balance between vagal and sympathetic influence on the SA node. Based on pharmacological data (Nyberg 1981) one would expect a consistently increasing LF:HF ratio with increasing exercise intensities. During light exercise (HR < 100 BPM) this would be due to vagal withdrawal and at higher intensities (HR >100 BPM) due to sympathetic activation (Robinson *et al.*

1966; Nyberg 1981). Due to the mathematical nature of the LF:HF ratio, the causes of any change would of course, not be obvious.

From the discussion of HF, HFnu, LF and LFnu the commonly observed pattern of change in LF:HF should be obvious. In certain cases LF:HF does increase at the onset of (light) exercise (Bernardi *et al.* 1990; Perini *et al.* 1990; Rimoldi *et al.* 1992; Breuer *et al.* 1993). Most commonly it then decreases as the exercise becomes more intense; it often shows a significant, sharp increase at very high or maximal exercise intensities. In some cases LF:HF drops at the onset of exercise (Arai *et al.* 1989). Only one study has found LF:HF to increase at high exercise intensities (Yamamoto *et al.* 1991).

With the exception of the work of Yamamoto *et al.* (1991) who give no explanation for their disparate findings, it is clear that LF:HF is a poor marker of sympathovagal balance during exercise. Most importantly it behaves in an almost reverse manner to that expected. The ratio also displays great variation between studies and within subjects in a single study. It is therefore an unsuitable maker of sympathovagal balance (Goldberger 1999).

7.1.4. *Alternative methodologies.*

Due to the above problems associated with GSA of RR interval data a number of alternative data treatments have been used in attempts to gain insight into the autonomic control of HR during exercise. A common approach is to use coarse graining spectral analysis (CGSA) instead of GSA. Proponents of this method (Yamamoto and Hughson 1991; Yamamoto *et al.* 1991; Yamamoto *et al.* 1992) have successfully shown patterns of behaviour in the high (parasympathetic indicator) and low (sympathetic indicator) frequency spectral bands which concur with current theory of sympathovagal interaction during exercise.

In addition to observing the harmonic (low and high) components of HRV, CGSA by the nature of its mathematics separates the non-harmonic component

of the RR interval data and allows analysis of this component. It has been shown that the high complexity of this chaotic or fractal component observed at rest, decreases during exercise as the RR intervals become more uniform. This has been used as a marker of sympathetic predominance (Yamamoto and Hughson 1994) and validated using data from autonomic blockade (Yamamoto *et al.* 1995; Hagerman *et al.* 1996).

This method is however, not without its problems. Gregoire *et al.* (1996) found that CGSA was unable to identify expected differences in autonomic control between trained and untrained individuals. This was in spite of clear differences in RR interval length at absolute exercise workloads. Based on this work Gregoire *et al.* (1996) and Myslivecek *et al.* (2001) have stated the subtraction of the non-harmonic component from the total power spectra increases the sensitivity of the PNS indicator (High/TP) to very small changes in HF. Gregoire *et al.* (1996) concluded that in their study, it was this large variability which made intergroup comparisons difficult.

Another alternative method to GSA includes the use of Poincare plot analysis. By plotting each interbeat period as a function of the previous one a two-dimensional vector analysis can be carried out (Brennan *et al.* 2001; Brennan *et al.* 2002). This method gives estimates of both instantaneous and long-term RR interval variability (Kamen and Tonkin 1995). Additionally, qualitative analysis of Poincare plots has led to the identification of several distinct patterns, known to be representative of certain conditions and pathologies (Bergfeldt and Haga 2003). This geometric method has the advantage of being largely independent of spectral power, and has been used successfully during exercise (Tulppo *et al.* 1996; Tulppo *et al.* 1998). It has, however, been stated that Poincare analysis may offer little more information than simple time domain measures of HRV (Carrasco *et al.* 2001).

7.1.5. *Availability of different heart rate variability analyses.*

Numerous methods to analyse RR interval data are available and the availability of commercial systems to carry out this analysis is also increasing. The analysis available within each system will in some way, determine how the RR interval data are treated by the researcher. For example, most commercially available analysers which designed for short-term ECG recording and analysis are capable of GSA via either autoregressive modelling or fast Fourier transformation. Frequency domain analysis is usually accompanied by standard time domain measures suitable to extract from short-term recordings. Few analysers are capable of CGSA, in fact one manufacturer (Advanced Medical Diagnostics Group Ltd. Leeds, UK) recently removed this function from its commercial software (VariaCardio, TF5) during updating.

To use GSA during exercise, modifications to the existing recommendations (Camm *et al.* 1996) for resting HRV measurement need to be made. Warren *et al.* (1997) attempted to validate several, modified versions of GSA as markers of sympathetic and parasympathetic activity during exercise using autonomic blockade. They noted that several previously reported techniques had yielded results that 'coincide nicely' with the established theories regarding autonomic response to exercise. However, none of these techniques had been microneurgraphically or pharmacologically validated.

Warren *et al.* (1997) used a modified version of HF (0.1 – 1.0 Hz) which encompassed all breathing frequencies observed during exercise. This was found to be a valid measure of vagal activity during exercise and muscarinic blockade (glycopyrrolate). They found a modified version of LF (0.004-0.1 Hz) divided by TP (0.004 – 1.0 Hz) to be an equivocal index of sympathetic activity based on exercise and β_1 -adrenergic blockade (esmolol) data.

The primary aim of the present study was to attempt to recreate these data using frequency bandwidths permissible by commercially available analysers. This is

because software in such analysers is commonly set to conform to current recommendations or is only variable within a specific range. Two commercially available analysers with adjustable frequency bandwidths were explored. From this, the bandwidths for use in the present study were determined.

The secondary aim of the study was to elucidate whether the new GSA framework could provide insight into the differential responses to exercise of fit and unfit subject groups determined by their $\dot{V}O_{2\max}$. This way the logical validity (Baumgartner 1989) of the new measures could be assessed. A number of differences between fit and unfit subjects would be expected.

Briefly, at rest it would be expected that fit subjects would display greater vagal activity compared to less fit counterparts. It is known that transfer to the upright position is commonly accompanied by vagal withdrawal and sympathetic activation (Jasson *et al.* 1997; Furlan *et al.* 2000). There is some evidence to suggest that adjustment to orthostatic stress (standing) and increased HR during low intensity exercise is the result of greater vagal withdrawal and reduced sympathetic activation in fit subjects (Winder *et al.* 1978; Portier *et al.* 2001; Myslivecek *et al.* 2002). At higher exercise intensities fitter subjects display a lower level of sympathetic activation compared to less fit subjects (Hughson *et al.* 1977; Kingwell *et al.* 1994).

On this basis it was hypothesised that the more fit subjects would have a higher vagal indicator value at rest and that expected decreases in this value during standing and exercise would be larger. It was hypothesised that increases in the sympathetic indicator during exercise would be greater in the unfit group. It was also hypothesised that these may occur at lower exercise intensities in the less fit subjects compared to the fit group.

7.2. Methods.

7.2.1. Subjects.

Twenty nine university staff and students (20 males median age 39 range 19 - 63) and nine females median age 35 (range 19 - 56) were included in the study. All subjects were healthy, defined as being free from illness at the time of testing. None were known to be taking any medication or have any cardiovascular problems that may have influenced the tests carried out. All subjects volunteered to participate in the study. Written, informed consent was given separately for each part of the study: resting HRV measures, exercise testing and blood lactate testing. Subjects were, therefore, able to participate in the study without consenting to blood sampling if they so chose. Complete blood lactate analysis was only completed on 20 subjects. One subject was excluded from the HRV analysis due to technical failure. Full HRV data during exercise was not collected on a large proportion of the subjects. This will be discussed further in the results and limitations sections.

7.2.2. Equipment.

7.2.2.1. Heart rate variability measurement.

HRV measures were made simultaneously using two instruments simultaneously; at rest and during exercise. Instrument one was a CardioPerfect ST 2001 HRV module (Cardio Control, Delft, The Netherlands). The system used a standard 12 lead ECG with a sampling frequency of 1000Hz. The sampling time for each HRV analysis was set at 5-min in agreement with current guidelines (Camm et al. 1996). The signal was digitised directly and the full ECG trace was shown in real time on a computer screen, this signal was simultaneously stored on the hard drive of a PC (Dell Computers, Texas, USA.) for post hoc analysis. Each sampling period was stored as a single patient ECG record. To analyse the variability in RR interval data an automated protocol within the HRV module

software was used. The automated filtering system is adjustable, and a standardised configuration was used which treated the data as described below.

Editing of the raw RR interval data was carried out using an automatic-threshold-detection algorithm. The software rejected RR intervals which differed by more than 20% from the previous interval. This interval was then interpolated with an interval generated on the preceding interval data. The remaining analysis was based on the corrected data file of 'normal' RR intervals. The RR data were then passed through a Hanning type window to remove baseline trends. The RR interval time series was decomposed to the frequency domain via fast Fourier analysis. The resulting power spectrum was divided into the following spectral bands HF (0.15-0.40 Hz) LF (0.04-0.15 Hz) and VLF (0.0033-0.04Hz). In addition to this time domain analysis was also carried out to give the following values RMSSD, SDNN and RR interval.

The second HRV analysis instrument was a VariaCardio (TF5) HRV analysis system (Advanced Medical Diagnostics Group Ltd. Leeds, UK). This system used a purpose built chest-strap with two electrodes, placed either side of the subject's heart. The chest strap sampled at rate of 500 Hz. This signal was then sent to a receiver and digitised. The ECG trace and a graphical representation of RR interval were displayed in real time on the screen of a designated portable computer (IBM Systems USA). The sampling period for each measurement was set at 300 seconds or 300 beats whichever was longer. The instantaneous HR was derived from the identification of QRS complexes which was sampled at a higher rate of 1000 Hz. The RR interval and full ECG data were both stored on the hard disk of the designated portable computer prior for post hoc analysis.

Stored RR interval data were then edited automatically by the software. The manufacturers do not give details of the algorithm used to edit the data. To ensure effective editing all data were manually edited by a single researcher. This was done by visually analysing the raw ECG trace and the graphical representation of the RR interval data given. The researcher rejected intervals

which appeared to represent potential artefacts, interference or noise. This filtered data set was then stored on the hard disk of the PC as a separate data file labelled filtered data. All further analysis was carried out on this filtered data set. The RR interval time series were decomposed to the frequency domain via fast Fourier analysis. The resulting power spectrum was divided into the following spectral bands HF (0.15-0.40Hz) LF (0.04-0.15Hz). Time domain analysis was also carried out to give the following values mean squared standard deviation (MSSD), SDNN and NN interval. The MSSD was then transformed to the RMSSD manually.

The reasons for the simultaneous use of two HRV systems were twofold. Firstly the dual analysis protocol was used to maximise the possibility of capturing the maximal amount of data. This is because it is known, especially during exercise that changes in the signal-to-noise ratio may prohibit the spectral analysis of RR interval data (Gregoire et al. 1996). Secondly, although HRV analysis is now commonplace during exercise there are no data pertaining to the agreement between different analysis systems under such conditions.

7.2.2.2. Respiratory measurements.

The volume of expired air (V_E) and gas exchange measurements were made breath-by-breath, using a Medical Graphics CardiO₂ online breath-by-breath analysis system (Medical Graphics Corporation, St. Paul Minnesota, USA). Automated software from the same company (Breeze Suite) created a full set of nine-panel-plots (Wasserman et al. 1999) by which aerobic threshold could be detected. Blood lactate analysis was carried out using a YSI 1500 Sport lactate analyser (Analytical Technologies, Hanford House, UK).

7.2.3. Protocol.

7.2.3.1. The Assessment of $\dot{V}O_{2\text{peak}}$ and CPO_{peak}

Subjects visited the laboratory on a total of three occasions to undertake different parts of the testing protocol. During visit one $\dot{V}O_{2\text{peak}}$ and CPO_{peak} were determined for each subject. Each subject completed an incremental exercise test (Bruce) on a motor-driven treadmill (Cardio Control, Delft, The Netherlands) to volitional exhaustion. This allowed an estimate of $\dot{V}O_{2\text{peak}}$ which was chosen in preference to true $\dot{V}O_{2\text{max}}$. $\dot{V}O_{2\text{peak}}$ has been found to be simple to measure, enduring standard of aerobic fitness (Mancini et al. 2000). It is less physically stressful than $\dot{V}O_{2\text{max}}$ for the subject and is therefore useful when using volunteers from clinical populations and the general population. Many subjects within these categories frequently have difficulty attaining true $\dot{V}O_{2\text{max}}$ as defined by current guidelines (ACSM 2000). During the test \dot{V}_E and O_2 and CO_2 exchange were monitored continuously.

After at least 40 min rest the subjects performed a constant maximal workload exercise test on the treadmill. In this instance the researcher manually controlled the speed and gradient of the treadmill to elicit a $\dot{V}O_2$ close to (within 10%) the value achieved during the Bruce test. Cardiac output (CO) was then assessed using the CO_2 rebreathing technique (Defares 1960). Cardiac power output (CPO) was subsequently calculated using CO and blood pressure measured by a hand-held aneroid sphygmomanometer).

Two non-invasive methods are available to assess cardiac output the equilibrium method (Collier 1955) and the exponential method (Defares 1960). The exponential method was chosen as subjects experience significantly fewer adverse side effects compared with the equilibrium measure (Vanhees et al. 2000). There are data to support both the validity (Marks et al. 1985; Kuji et al. 1991) and reliability (Marks et al. 1985) of this method. Briefly, during the constant maximal workload test the subject's air supply was changed from room

air to breathing from a bag containing 5% CO₂ and 14% O₂ for 10-20 s. The subject then rebreathed from this bag until a plateau in the increase in CO₂ concentration was observed. The increase in CO₂ and plotting of the CO₂ concentration during the manoeuvre was carried out by an automated protocol within the BreezeSuite software.

7.2.4. Heart rate variability measurement.

During the second visit, each subject's resting HRV was analysed. In accordance with current guidelines for the capture of RR interval data for HRV analysis subjects attended the laboratory having refrained from eating or smoking for 2 hours. Subjects were also asked to refrain from alcohol or caffeine containing beverages and exercise on the day of the test, and exercise and heavy alcohol consumption on the evening prior to the test. Where subjects had failed to meet the requirements of the protocol an assessment was made on the potential impact of their behaviour on HRV measures and where necessary the test was rescheduled.

Disposable electrodes (Blue Sensor Medicotest, Olstykke, Denmark) were placed in the standard configuration for 12-lead ECG and the TF5 chest strap was placed laterally across the subject's heart in accordance with manufacturer's instructions. Subjects were then asked to lie on a bed in a quiet laboratory (18-22 °C). Subjects were asked to relax and the researchers monitored their HR visually until it became stable. Two simultaneous 5-min ECG recordings were then made. Two researchers were required to synchronously start both pieces of equipment. Although some auditory clues may have been available, the subject was purposely not informed of the start of the recording period. At the end of the first recording the subject was asked to stand with feet shoulder width apart with hands placed on the back of a tall stool. Subjects were asked not to 'shuffle' or transfer their weight laterally during the data collection period. A second 5-

min ECG recording was made when the subjects HR was deemed to be stable following the change in position.

7.2.5. Measurement of HRV, blood lactate and CPO during incremental exercise.

At the third visit to the laboratory, consenting subjects were first required to give a resting blood lactate sample. An arterialised capillary finger prick sample was taken from the index finger of the left hand and the whole blood was then transferred via micropipette to the YSI 1500 Sport Lactate analyser. All data were recorded from the analyser immediately and stored electronically for later analysis.

Subjects were then required to complete three stages of incremental exercise (25, 50 and 75% $\dot{V}O_{2peak}$). During this test ECG was continually monitored and breath-by-breath analysis of expired air was carried out using the techniques already described. The intensity of this exercise was determined from the $\dot{V}O_2$ at each level. When the level of predetermined $\dot{V}O_2$ was reached, a further 5-min ECG sample was taken. During this period, a blood pressure measurement was taken. At the end of 5-min a blood lactate measurement was made using the procedure described previously. At the end of each stage the subjects performed the rebreathing manoeuvre described earlier but with the addition of being instructed to breathe in time with a metronome eliciting a breathing rate of 0.15 Hz. This was done in accordance with the manufacturer's instruction to ensure the adequate rebreathing of the reference gas mixture from the bag.

7.2.6. Data treatment.

Data were selected from $n = 29$ cases and placed into two groups according to the following criteria. Subject groups were designed to be of roughly equal size with an equal mean age although subjects were not age matched. Subjects were

then allocated to either a high fitness (HI) or low fitness (LO) group based on their relative $\dot{V}O_{2,peak}$ from the incremental treadmill test protocol described previously.

Each subject was required to have a full data set up to 50% $\dot{V}O_{2,peak}$. It was also required that resting HRV data had been collected on the same day as exercise data. Data from some subjects were then rejected on the basis of unsuitability (usually non-stationarity) of recorded tachograms. Those with an excessive number of interpolated beats (>20%) at exercise levels up to 50% $\dot{V}O_{2,peak}$ were also rejected from the analysis.

7.2.7. *Post-hoc heart rate variability analysis.*

The ECG data were stored on the hard disk of a computer (Dell Microsystems, Texas, USA) and HRV analysis was carried out as described previously except for the following data treatments.

The LF band was altered from the default and recommended (Camm *et al.* 1996) bandwidth of 0.04-0.15 Hz to 0.004-0.1 Hz. The HF bandwidth was altered from 0.15-0.40 Hz to 0.1 – 0.5 Hz. This was as close to the bandwidths used by Warren *et al.* (1997) for exercise HRV analysis as all software would allow.

Power in the modified HF band (ms^2) was then used as a marker of vagal activity. The marker of sympathetic activity used was the modified LF band (ms^2) divided by the total spectral power (0.004 – 0.50 Hz) or LF/TP.

7.2.8. *Statistical analysis.*

All values for RR interval, HF and LF were checked for normality of distribution using a Shapiro-Wilk's test. Where skewed distributions were found and data

were suitable, natural logarithms were taken and the data again checked for normality.

7.2.8.1. Analysis of complete data set.

Changes in HF and LF/TP were analysed from rest to 50% $\dot{V}O_{2\text{peak}}$ by repeated measures analysis of variance (rm-ANOVA). Data were checked for homogeneity of variances (Levene's test) and sphericity (Mauchly's test). Where either of these assumptions were not met, an appropriately adjusted alpha value was assumed. Differences between LF and HF from 50 – 75% $\dot{V}O_{2\text{peak}}$ were analysed by repeated measures *t*-test (rm *t*-test) due to missing cases in the latter condition. Further, *post hoc* analysis was also carried out using rm *t*-test.

7.2.8.2. Analysis of HI and LO fitness groups.

Differences in the behaviour of HF and LF/TP during exercise between the HI and LO groups were analysed by two-way mixed ANOVA with repeated measures (mixed ANOVA) from rest to 50% $\dot{V}O_{2\text{peak}}$. Differences between 50 and 75% $\dot{V}O_{2\text{peak}}$ were analysed using rm *t*-test. This was due, again, to the reduced number of cases with complete data sets at the latter intensity. *Post hoc* analysis was carried out by comparing values for HF and LF/TP in each condition with baseline values. At each intensity, HF and LF/TP were also compared with values from the preceding condition. This was done using either rm *t*-test or a Wilcoxon matched pairs *U*-test. A value of $P \leq 0.05$ was accepted as statistically significant.

7.3. Results.

From the 29 original subject cases, six were rejected because resting data were recorded on a separate occasion to exercise data. Data from four subjects were

found to be incomplete on all necessary measures up to 50% $\dot{V}O_{2\text{ peak}}$. The tachograms of two subjects were rejected due to significant non-stationarity.

Complete data were analysed on $n = 17$ subjects ($n = 8$ in the LO group, $n = 9$ in the HI group). There were two females in each group. Results of power analysis on the basis of the work of Warren *et al.* (1997) suggested this sample size would produce statistical power in excess of $\beta = 0.8$ at $P = 0.05$). However it should be noted that the sample size estimation carried out was at best crude, due to differences in study population and methodologies.

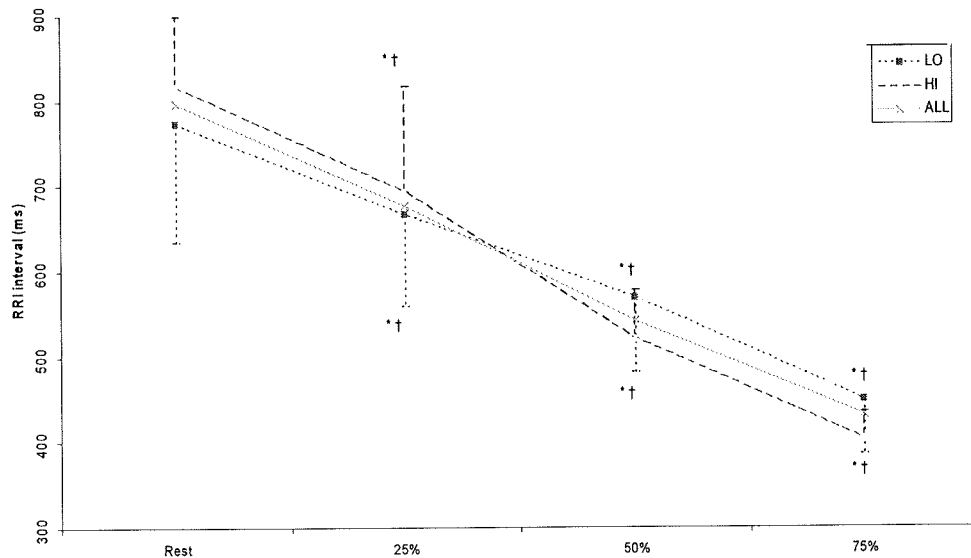


Figure 7-1 Changes (mean \pm SD) in RR interval during incremental exercise.

*** Significantly different from baseline. † Significantly different from preceding value.**

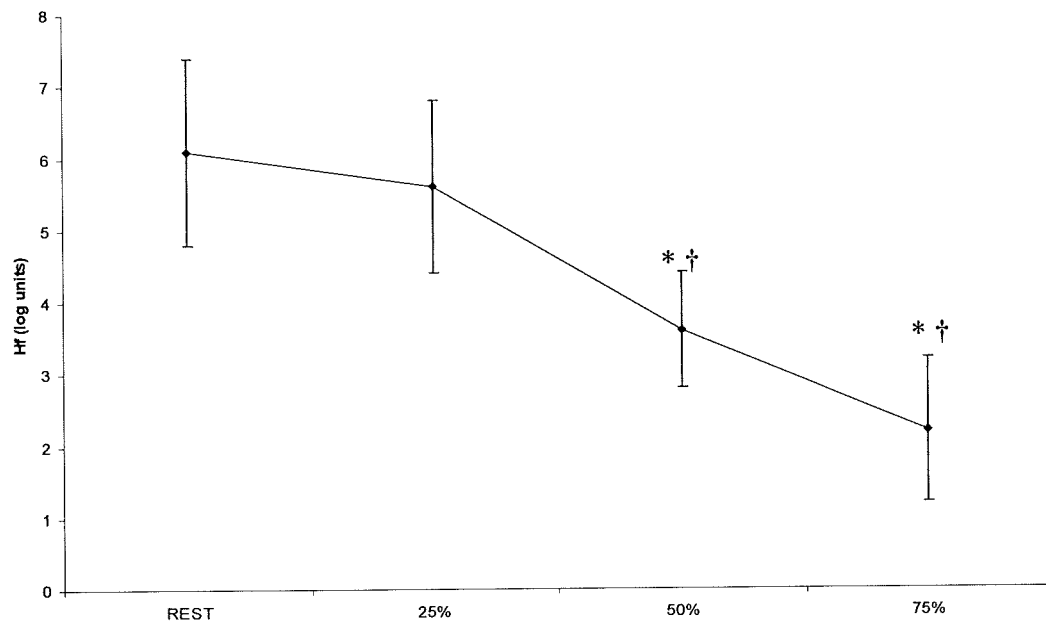


Figure 7-2 Changes (mean \pm SD) in HF power (0.1-0.5Hz) during incremental exercise.
 *Significantly different from baseline. † Significantly different from preceding value.

7.3.1. Whole group data.

Independent *t*-tests showed the groups to be similar in terms of height, weight and BMI. Non-parametric analysis (Mann-Whitney *U*-test) showed the groups did not differ significantly in age (Table 7-1). The only significant difference between the two groups was in $\dot{V}O_{2peak}$. This was shown to be greater in the HI group when expressed in absolute terms and also through non-parametric analysis when expressed in relative terms.

Of the 17 subjects entered into the final analysis data were not available at 75% $\dot{V}O_{2peak}$ in $n = 5$. Four of these subjects were in the HI fitness groups and only one in the low fitness group.

Table 7-1. Population characteristics of the LO and HI fitness groups.

	LO	HI	Significant ($P \leq 0.05$)
Age	45.0 ± 14.9	30.9 ± 11.7	No*
Height (cm)	174.4 ± 6.1	179.1 ± 8.2	No
Weight (kg)	79.1 ± 14.9	79.6 ± 10.9	No
BMI	25.9 ± 3.5	24.7 ± 2.2	No
$\dot{V}O_{2\ peak}$ ($l \cdot \text{min}^{-1}$)	2.3 ± 0.6	3.6 ± 5.3	Yes
$\dot{V}O_{2\ peak}$ ($\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	28.5 ± 3.8	45.4 ± 6.7	Yes*

All values are means \pm SD. *Non-parametric analysis used.

Figure 7-1 shows the RR interval data for all subjects and separately in the HI and LO groups. Mixed ANOVA with repeated measures carried out on the natural logarithms this data revealed a significant main effect of exercise intensity ($F = 115.1$, $P = 0.0001$).

There was a clear effect of exercise intensity on HF (Figure 7-2) which was reduced with increasing exercise intensities ($F = 38.1$, $P = 0.001$). HF decreased between each exercise intensity, *post hoc* analysis revealed the reduction in HF from rest to 25% $\dot{V}O_{2\ peak}$ was not statistically significant. Values of HF at 50 and 75% $\dot{V}O_{2\ peak}$ were significantly lower than those at preceding intensities and from baseline values.

The effect of exercise intensity of LF/TP was less pronounced (Figure 7-3). No values differed significantly from baseline. There was no change in LF/TP from rest to 25% $\dot{V}O_{2\ peak}$ ($t = 0.8$, $P = 0.44$). There was a clear increase in LF/TP from 25% to 50% $\dot{V}O_{2\ peak}$ which was close to statistical significance ($t = 1.7$, $P = 0.09$). The only statistically significant difference was a decrease from 50 – 75% $\dot{V}O_{2\ peak}$ ($t = 2.4$, $P = 0.04$).

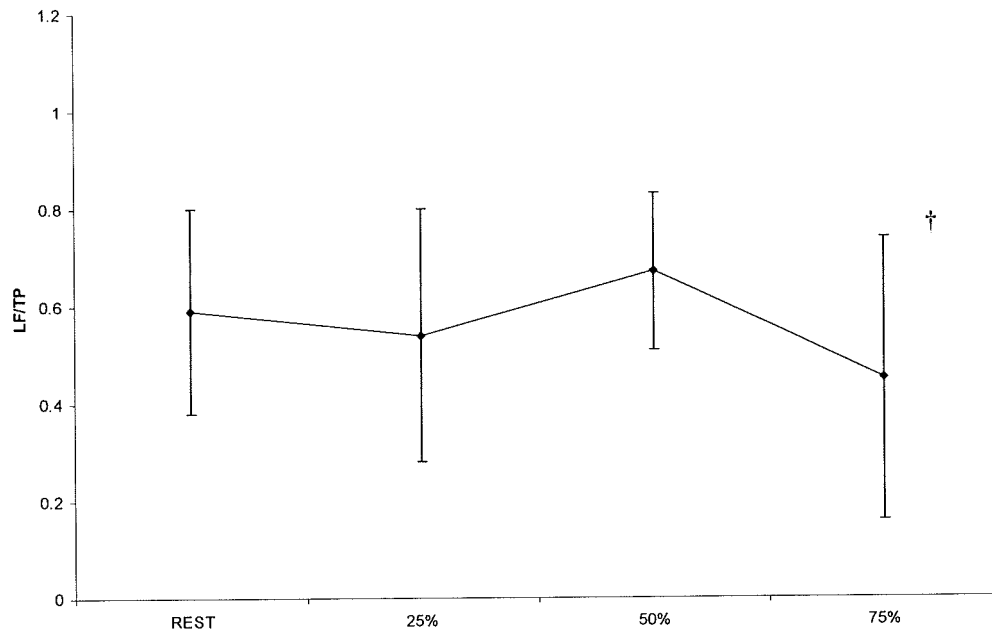


Figure 7-3 Changes (mean \pm SD) in LF/TP during incremental exercise.

*** Significantly different from baseline. † Significantly different from preceding value.**

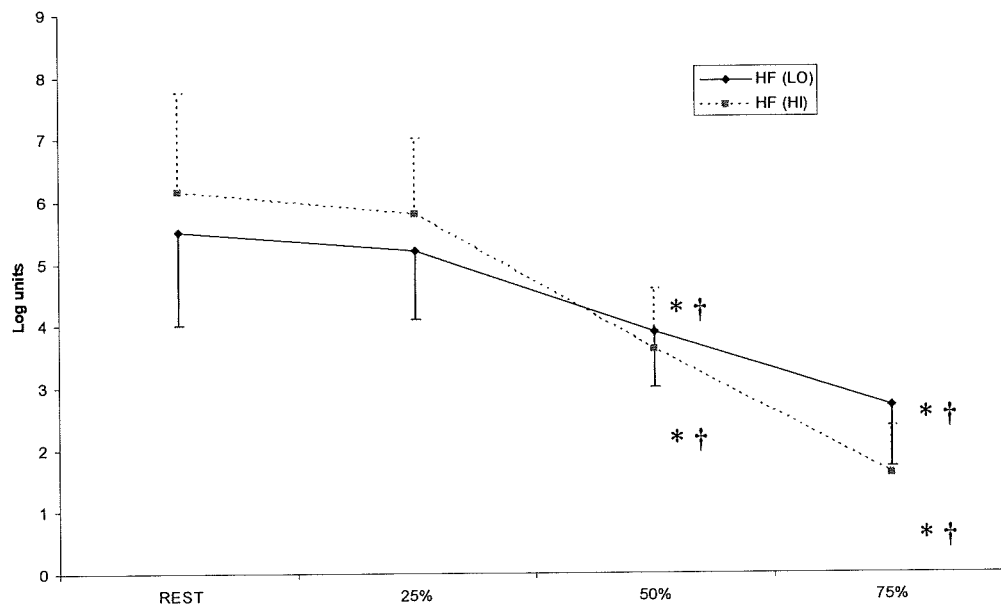


Figure 7-4 Changes (mean \pm SD) in HF during incremental exercise in HI and LO fitness groups.

*** Significantly different from baseline. † Significantly different from preceding value.**

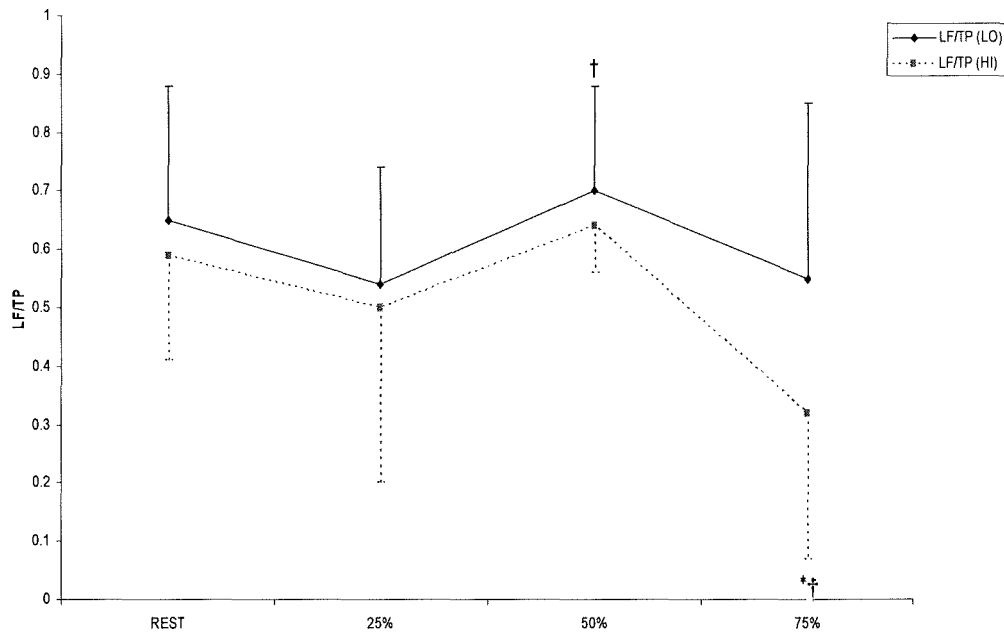


Figure 7-5 Changes (mean \pm SD) in LF/TP during incremental exercise in HI and LO fitness groups.

***Significantly different from baseline. † Significantly different from preceding value.**

7.3.2. LO and HI fitness groups.

When the subjects were divided into LO and HI fitness groups based on $\dot{V}O_{2\ peak}$ there was a group \times intensity interaction for HF (Figure 7-4) which was close to statistical significance ($F = 3.0, P = 0.07$). *Post hoc* analysis of both groups showed significant reductions in HF from 25-50% and from 50-75% $\dot{V}O_{2\ peak}$. The 50 and 75% $\dot{V}O_{2\ peak}$ values for HF were also significantly reduced from baseline values in both groups. Independent *t*-test showed that HF was not significantly different between groups in any of the exercise conditions.

There was no group \times intensity interaction for LF/TP (Figure 7-5). The LO group showed a trend toward increased values over all exercise intensities. There was a significant ($Z = 2.1, P < 0.05$) increase from 25-50% in the LO group which did not exist in HI. No values for LF/TP were found to differ significantly

from baseline. Independent *t*-tests showed no differences in LF/TP between groups at rest or during exercise.

7.4. Discussion.

The aim of the present investigation was to assess whether valid indexes of vagal and sympathetic activity could be generated through the manipulation of spectral HRV measures within the limits imposed by commercially available analysers. The results of the study demonstrate that HF power measured from 0.1- 0.5 Hz provides a good index of vagal activity during exercise. The ratio of LF (0.01 – 0.10Hz)/TP (0.01-0.5 Hz) was put forward as an index of sympathetic activity. Although some of the behaviour of this measure during exercise suggest it may be of some use as a maker of sympathetic activity the majority of the results suggest that this is at best, an equivocal marker. Additionally its use may be limited to lower ($<75\% \dot{V}O_{2\max}$) exercise intensities.

7.4.1. HF as a vagal index.

Warren *et al.* (1997) suggested the use of HF from 0.1-1.0 Hz as a measure of vagal activity during exercise. In the present study this was modified to allow data collection using commercially available analysers. These are limited in the spectral range over which RR intervals are analysed. The justification for altering the HF band for use in exercise study is that the centre of the HF peak is closely linked to respiratory frequency (Hayano *et al.* 1991; Ori *et al.* 1992; Brown *et al.* 1993; Casadei *et al.* 1996; Uusitalo *et al.* 1996; Malpas 2002). The recommended (Camm *et al.* 1996) frequency range for HF (0.15-0.40 Hz) may exclude some of the very low respiratory rates shown at rest by fit subjects and the high rates associated with (intense) exercise. By expanding the HF band, all respiratory-mediated vagal activity is included for analysis.

Warren *et al.* (1997) found their version of HF declined throughout incremental exercise intensities of 0, 50, 100 and 150 W demonstrating vagal withdrawal. This pattern of HF reduction corroborated the observed HR response. The validity of HF as vagal marker was further supported by pharmacological data. It was found that changes in HF were attenuated by both muscarinic and combined autonomic blockade. Changes in HR at low exercise intensities were not altered by β -adrenergic blockade (esmolol). HF was slightly lowered by esmolol but this was attributed to a small amount of, baroreflex-mediated vagal withdrawal that did not cause a significant tachycardia.

7.4.1.1. HF during exercise – replication of previous data.

In the present study autonomic blockade was not used. However, it was found that the small increase in HR from rest to 25% $\dot{V}O_{2\text{peak}}$ was accompanied by the smallest reduction in HF. The larger increase from 25-50% $\dot{V}O_{2\text{peak}}$ was mirrored by a large, statistically significant reduction in HF power. This would be expected as vagal withdrawal has been found to be the major cause of exercise induced tachycardia up to this intensity. From 50-75% $\dot{V}O_{2\text{peak}}$ HR continued to increase linearly but the reduction in HF was less pronounced, indicating that the majority of vagal withdrawal had already occurred.

When using HF (0.15 - 0.40 Hz) as markers of vagal tone during exercise a paradoxical increase in this measure is often found at higher exercise intensities (Tulppo *et al.* 1996; Tulppo *et al.* 1998) (Casadei *et al.* 1995). This has been attributed to increased respiratory rate and depth enhancing respiratory sinus arrhythmia. Bernardi *et al.* (1990) provided evidence for this and the contribution of this non-neural input was later quantified as approximately 60% during intense exercise (Casadei *et al.* 1995). Warren *et al.* (1997) found this to be the case in four of their ten subjects. In the present study, all subjects demonstrated a reduction in HF from 50 – 75% $\dot{V}O_{2\text{peak}}$. The mean group reduction was 1.6 log units from 3.8 to 2.2 ($t = 5.1$, $P < 0.001$).

7.4.1.2. Logical validity of HF as a vagal index.

Warren *et al.* (1997) verified the validity of their measure of HF using β^l -adrenergic, muscarinic and combined autonomic blockade. In the present study the logical validity of HF was determined by splitting the subjects into two groups of high fitness (HI) and low fitness (LO). The differences in HR response to exercise and the relationships between HR and HF in the two groups were then compared. The HI fitness group demonstrated a longer RR interval at rest compared to the LO group (Figure 7-1). The longer RR interval in HI was maintained from rest to 25% $\dot{V}O_{2\ peak}$. HF was elevated at rest in HI compare to LO and remained so at 25% $\dot{V}O_{2\ peak}$. The changes in HF were very similar between these two conditions in both groups. From 25-50% $\dot{V}O_{2\ peak}$ the decrease in RR interval was much more marked in HI. Similarly, the reduction in HF, although significant in both groups was much greater in HI. This fits with current theory by suggesting a greater proportion of the exercise-induced tachycardia observed was due to vagal withdrawal in the fitter subjects. From 50-75% the decreases in RR interval in LO and HI were very similar. The decrease in HF in the LO group was statistically significant ($t = 2.54, P = 0.04$), however the decrease in the HI group was of a much greater magnitude ($t = 13.7, P < 0.001$) and showed less within-subject variation. This may indicate that at this higher exercise intensity vagal withdrawal may still be occurring, and, to a greater degree, in the fit subjects. This reaction is much less evident in the LO group.

Although it is evident from Figure 7-3 that HF at 50 and 75% $\dot{V}O_{2\ peak}$ is actually higher in LO it should be noted that HI were working at higher absolute workloads. This difference is therefore expected. Finally the large standard deviations evident in the LO group at the 75% level indicate a varied response to changes in exercise intensity, whereas the very smaller spread of scores in the HI group suggest a more uniform reaction. The meaningfulness of this result is as yet unclear.

7.4.2. *LF/TP as a sympathetic index.*

Warren *et al.* (1997) found LF (0.004-0.1 Hz) / TP (0.004-1.0) to be an equivocal marker of sympathetic activity. The ratio was only found to increase significantly from baseline at exercise intensities of 150 and 200 W. This finding fits well with data concerning sympathetic activation and its role in exercise HR. Current theory suggests that increased sympathetic activity only plays a major role during exercise eliciting a HR greater than 100 BPM.

7.4.2.1. LF/TP During exercise – replication of previous data.

In the present study LF/TP remained unchanged from rest to 25% $\dot{V}O_{2\text{peak}}$ and then increased ($P = 0.063$) from 25-50% $\dot{V}O_{2\text{peak}}$. This again fits with theory regarding sympathetic activation. From rest to 25% $\dot{V}O_{2\text{peak}}$ RR interval decreased from 800 to 680 ms (80 – 90 BPM). From 25% - 50% $\dot{V}O_{2\text{peak}}$ the decrease was 680 – 550 ms (90 - 120 BPM). It is therefore, between 25 and 50% $\dot{V}O_{2\text{peak}}$ that an initial increase in sympathetic activity would be expected. The present data support this notion. From 50-75% $\dot{V}O_{2\text{peak}}$ Warren *et al.* (1997) showed a less marked increase in LF/TP than the previous increment. In the present study LF/TP actually showed a significant decrease from 50-75% $\dot{V}O_{2\text{peak}}$. This would seem to invalidate it as a measure of sympathetic activity. However, several methodological issues must be addressed before LF/TP is dismissed. Firstly, data were only present on 70% of subjects at this level. Of this fraction, 88% of the LO group had data present. In the HI group this value was only 44%. This lack of data was due mainly to low signal:noise ratios present at 75% $\dot{V}O_{2\text{peak}}$. In many of the data sets which were present, a large proportion of the RR intervals were interpolated. It may therefore be wise to treat data obtained for LF/TP (and HF) at 75% $\dot{V}O_{2\text{peak}}$ with some caution. Lastly, the behavior of LF/TP was not uniform between subjects. Whereas most subjects showed a

decrease in LF/TP from 50-75% $\dot{V}O_{2\text{peak}}$, one subject showed a very marked increase and two others showed smaller increases.

7.4.2.2. Logical validity of LF/TP as a sympathetic index.

When the data were split by subject fitness level (LO and HI) there was no interaction between groups across the exercise conditions. A clear trend of elevated LF/TP in LO was, however, evident. Values for LF/TP were higher at rest and remained elevated throughout all exercise intensities. From 25-50% $\dot{V}O_{2\text{peak}}$ there was a significant increase in LF/TP from baseline values in the LO group only. This change was coincidental with the point at which the HI group demonstrated a greater reduction in HF. This reduction was also accompanied by a more marked decrease in RR interval in HI compared to LO. This can be interpreted as inferring that from 25-50% $\dot{V}O_{2\text{peak}}$ subjects in the HI group increased their HR by vagal withdrawal whereas the less fit (LO) subjects were more reliant on sympathetic activation to increase HR. As mentioned previously, relative workloads were assigned to each group based on previous maximal $\dot{V}O_{2\text{peak}}$ values. Because of this fact, the absolute workload and size of the work increment in the HI group was much greater.

It seems LF/TP may be useful maker of sympathetic activity within the range of resolution of the recording system or at lower exercise intensities. Until the former is clearly defined it remains to be determined as to which of these two possibilities is the limiting factor in the use of this measure.

7.4.2.3. Problems with LF/TP.

Warren *et al.* (1997) concluded that LF/TP was at best an 'equivocal' maker of sympathetic activation based on its behavior during exercise. Closer examination of their results reveals that from 0 – 150 W LF/TP increased constantly, the lack of statistically significant differences found are probably due

to the small ($n = 10$) sample size. The second reason for rejection of LF/TP comes from the data achieved under pharmacological blockade and is more convincing. Under β -adrenergic blockade LF/TP showed quantitatively similar increases as in the placebo condition. LF/TP also increased at 50 W, a workload at which β -adrenergic blockade failed to attenuate HR response. Both these scenarios show a clear uncoupling of the activity of LF/TP and sympathetic nervous outflow.

In the present study it seems that LF/TP may be a similarly equivocal marker to the ratio described by Warren *et al.* (1997). Prior to any further investigation, caution must be used in assuming LF/TP is a marker of sympathetic activation due merely to the fact that its behavior is coincidental with current physiological theory. The fact that LF/TP here and previously described (Warren *et al.* 1997) bears some relationship to the expected pattern of behavior during exercise warrants further investigation. It is an obvious improvement on LF (0.04-0.15 Hz) which has been used previously in raw and normalised units to indicate sympathetic activity during. When LF power (ms^2) is measured during exercise a large decrease in spectral power is commonly observed at the onset of exercise (Furlan 1987; Rimoldi *et al.* 1990; Rimoldi *et al.* 1992). LF then continues to decrease with greater exercise intensities (Arai *et al.* 1989; Bernardi *et al.* 1990; Breuer *et al.* 1993). To control for the overall reduction in spectral power observed investigators have used LFnu or LF:HF to represent sympathetic activity during exercise. The use of these ratio has lead to similarly paradoxical results with large decreases in LFnu and LF:HF reported during exercise, especially at higher exercise intensities (Perini *et al.* 1990; Breuer *et al.* 1993; Perini *et al.* 1993; Casadei *et al.* 1995; Perini *et al.* 2000; Perini *et al.* 2002).

There is evidence to suggest that LF is at least, in part mediated by vagal activity (Akselrod *et al.* 1981; Vybiral *et al.* 1990; Cacioppo *et al.* 1994; Jokkel *et al.* 1995) the overall decline in HF and TP must be controlled for and a ratio is the

only option to do this it may simply be a question of which ratio is used and within which frequency bands the numerator(s) and denominator(s) lie.

7.5. Conclusions and limitations.

Data from the present study support the use of HF (0.1-1.0 Hz) as an index of vagal activity during exercise. They also partially support the use of LF/TP as a sympathetic index. Trends toward differences between fit and unfit subjects in these indices demonstrate their logical validity and further support their use. The lack of many statistically significant differences between groups was probably due to the small sample size employed. Although sample size calculations were made, they were based on findings using different vagal and sympathetic makers. This highlights a real problem when undertaking novel research of this type. The fact that calculations were attempted at all is an advance on much of the previous data in this field. Furthermore, the present data may now be used to provide sample size estimates for further investigation. Investigating the logical validity of these methods using absolute workloads would facilitate comparison of findings made using these ratios with those published previously (Warren *et al.* 1997). This may elucidate more clearly, differences in vagal and sympathetic activity between fit and unfit subjects during exercise. It is of note that non-invasive makers of sympathetic activation are available. Both baroreflex function testing and other applications of beat-to-beat blood pressure monitoring are well established as makers of sympathetic activation (Uusitalo *et al.* 1996) and further work may be directed at improving the understanding and application of these methods and quantifying the relationships between them and potential markers of sympathetic activity from HRV.

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CHAPTER 8. HEART RATE VARIABILITY AND TRAINING INDUCED-BRADYCARDIA: INFERENCE FROM META-ANALYSIS.

Abstract

Chronic exercise training produces a resting bradycardia which is thought to be due, in part to increased vagal modulation. The aim of the present study was to determine the cross-sectional and longitudinal effects of exercise training on heart rate and measures of heart rate variability associated with vagal cardiac modulation and to quantify the relationship between changes in these measures.

Random effects models of effect size (d) for change in high frequency (HF) power, standard deviation of normal to normal intervals (SDNN) and RR interval were calculated. Within-group heterogeneity was assessed using the Q statistic. Where heterogeneous effects were found subgroup analyses were performed using the between-group Q statistic.

Cross-sectional differences between sedentary and active subjects were significant for all measures ($P < 0.0001$) and heterogeneous. Subgroup analysis revealed the level of athletic training (recreational vs. elite athletes) significantly impacted on effect size magnitude. Longitudinal analysis showed significant effects of exercise training on HF ($d = 0.48$, C.I. $0.26 - 0.70$, $P = 0.00003$), SDNN $d = 0.827$ (C.I. $0.36 - 1.30$, $P = 0.001$) and RR interval ($d = 0.75$, C.I. $0.51 - 0.96$, $P < 0.00001$). Effect sizes for SDNN and RR interval data were significantly heterogeneous and subgroup analysis revealed significantly smaller responses of SDNN and RR interval to training in older subjects ($P < 0.1$). Factors such as subject sex and length of training intervention also modulated effect size. Effect sizes for change in HF were homogenous, although there was a trend towards an attenuated response to training in older subjects ($P > 0.1$). Serial curve estimation (linear, quadratic, cubic) revealed strong associations between HRV measures and change in RR interval in cross sectional differences, but only weak

($P > 0.05$) relationships between effect sizes for change in HF, SDNN and RR interval.

Exercise training results in a significant increase in RR interval duration, SDNN and HF power. These changes are influenced by the age of the study population. The smaller effect size for HF and the weak relationship between these measures suggest factors additional to increased vagal modulation are responsible for training bradycardia.

This chapter, in truncated form has been published in *Medicine and Science in Sports and Exercise*, see appendix IV.

8.1. Introduction.

The aim of the present study was to assess the chronic effects of exercise training on resting heart rate (HR) and overall variability in HR standard deviation of normal-to-normal intervals (SDNN) and high frequency spectral power (HF).

Although a slower resting HR is an accepted phenomenon in athletes (Raab *et al.* 1960; Frick *et al.* 1967; Ekblom *et al.* 1973; Scheuer and Tipton 1977) the exact cause is unclear. Data from selective and dual pharmacological blockade have claimed to support the notion that resting bradycardia in athletes is the result of a relative increase in vagal tone (Raab *et al.* 1960; Frick *et al.* 1967; Ekblom *et al.* 1973; Scheuer and Tipton 1977; Williams 1985; Smith *et al.* 1989). In a number of cases, data from such studies (Raab *et al.* 1960; Ekblom *et al.* 1973) are open to alternative interpretation (Katona and Jih 1975) or have methodological shortcomings (Frick *et al.* 1967). Incongruent results also exist (Maciel *et al.* 1985). Additionally, the dual blockade method has produced evidence to suggest that decreased intrinsic firing rate of the SA node may be responsible for the bradycardia observed following physical training in humans (Sutton *et al.* 1967; Lewis *et al.* 1980) and animals (Hughson *et al.* 1977).

Despite wide acceptance of increased vagal tone as the cause of bradycardia, there are little experimental data to support this view (Frick *et al.* 1967; Katona and Jih 1975). Quantification of the role of intrinsic and vagal influences on bradycardia is, therefore, difficult (Maciel *et al.* 1985).

Non-invasive methodologies based on beat-to-beat variation in HR have been used to estimate the vagal contribution to bradycardia (Katona and Jih 1975) and have produced results supporting increased vagal tone (Kenney 1985). Early studies used simple, time domain analysis by measuring the amplitude of change in RR interval during respiration (Respiratory Sinus Arrhythmia, RSA). The modulation of rhythmic oscillations in RR interval during the respiratory cycle is known to be vagally mediated (Pomeranz *et al.* 1985; Hayano *et al.* 1991;

Malliani *et al.* 1991; Hedman *et al.* 1992; Badra *et al.* 2001). Therefore, greater RSA amplitude has been used to indicate an increased vagal tone (Melanson 2000). These studies have shown training-induced bradycardia to commonly be accompanied by greater RSA. On this basis, bradycardia is assumed to be vagally mediated.

The measurement of rhythmic oscillations in RR interval has been developed further to provide a large number of measures in both the time and frequency domains. The analysis of heart rate variability (HRV) is now, recognised as a non-invasive measure by which resting autonomic (particularly vagal) nervous activity can be assessed (Pagani *et al.* 1986).

At rest, it is assumed that the majority of short-term oscillations in HRV are predominantly under vagal mediation. On this basis, simple time domain measures of overall RR interval variability have been proposed as useful vagal makers. One commonly used 'global' HRV measure is the standard deviation of normal-to-normal intervals (SDNN). This is sometimes reported as SDRR (the standard deviation of RR intervals). The use of SDNN is more correct as raw ECG signals (containing RR intervals) are often contaminated with ectopic beats and recording artefacts. These can influence HRV measures significantly and are commonly removed by combinations of automated beat-rejection algorithms and manual data editing. The resulting time series is commonly referred to as normal-to-normal (NN) interval data. Therefore, in the remainder of this chapter SDNN will be used regardless of the nomenclature employed by cited authors.

Spectral analysis of HRV provides information concerning rhythmic oscillations in RR interval at a number of frequencies. The most clearly defined of these are the high frequency (HF) oscillations between 0.15 – 0.40 Hz that commonly display a peak in spectral power concurrent with the centre of the respiratory frequency. There are many data to suggest that these HF oscillations are almost entirely vagally mediated (Akselrod *et al.* 1981; Pagani *et al.* 1986; Camm *et al.* 1996).

To elucidate whether exercise training is associated with significant resting bradycardia a meta-analysis of cross-sectional studies comparing athletes and active subjects with controls was carried out. In an attempt to infer causality from such findings, a further analysis of exercise intervention studies was also completed. In order to determine the degree to which any observed bradycardia was vagally mediated meta-analyses of SDNN and HF were carried out. To quantify the strength of any relationship between changes in RR interval, SDNN and HF correlational analysis was undertaken. To explain any heterogeneity, subgroup analyses were also completed.

8.2. Methods.

8.2.1. Search strategy.

The Pubmed and Ovid databases were searched using the MeSH terms 'heart rate variability' and: 'exercise' 'activity' 'athlete(s)'. A second search using the terms: 'bradycardia' and 'autonomic control' in conjunction with both previous search terms was also performed. Full text articles were then obtained and the bibliographies were searched manually to obtain further studies not identified electronically.

8.2.2. Criteria.

Only studies published in the English language and conducted in healthy persons of at least 18 years old were included. For longitudinal comparisons, an aerobic exercise intervention of at least four weeks duration was required. The use of a control group was originally an inclusion criterion but was later revoked. However, where a control group was present, the use of randomised group allocation remained a criterion. All studies were required to have measured either HF or SDNN.

Where a trial meeting the above criteria had assessed HRV across an exercise intervention, the data were further investigated to see if changes in RR interval had also been assessed. Failure to report change in RR interval was did not mean the study was excluded from the HRV meta-analysis. However, only studies reporting HRV measures and RR interval data concurrently were entered into the meta-analysis of changes in RR interval.

8.2.3. *Review process.*

The searches lead to the identification of 67 potential studies for inclusion in both analyses. The MeSH terms used provided many irrelevant studies due to the commonality of the terms used and their combinations. Studies were assessed in terms of the inclusion criteria by a single author. After application of the inclusion criteria only 47 of the studies identified were investigated further. From herein, the inclusion criteria for each type of comparison and HRV measurement analysed were different and are therefore addressed separately below.

8.2.4. *Analysis of data from cross-sectional study design.*

8.2.4.1. High frequency power.

The measure of HRV used was required to be high frequency spectral power (HF). It was deemed necessary that this had been derived from either autoregressive, Fourier type transformation or reported as the harmonic component from coarse graining spectral analysis. These data were required to be presented in raw units (msec^2) or transformed units. Normalised units ratios and percentages of total power were not used in the analysis.

From the 67 potential studies gained from the search criteria, 27 studies were identified as being useful, cross-sectional comparisons of HRV between groups.

On further investigation of these studies, four were rejected as neither HF nor SDNN were used as measures of HRV (Katona and Jih 1975; Kenney 1985; Reiling and Seals 1988; Melanson 2000). Where HF was reported, the results of two studies were rejected as only normalised units were given (Shin *et al.* 1995; Shin *et al.* 1997). In another, the HF (msec²) was given in a format from which it was not possible to calculate effect size (Macor *et al.* 1996). Following preliminary analysis of the data one further study was rejected due to the inclusion of an unsuitable control group (Sacknoff *et al.* 1994). Seventeen studies with a total of 20 trials were entered into the final analysis.

8.2.4.2. SDNN

Two studies from which the reported HF data were used in the above analysis failed to report SDNN. No additional studies were added to those included in the above section for HF. In all, 12 studies with a total of 15 trials were entered into the final analysis.

8.2.4.3. RR interval.

Raw RR interval data and differences between groups were available in all studies and all trials included in the analysis of both HF and SDNN. Therefore, 17 studies with a total of 20 trials were included in this analysis

8.2.5. Analysis of data from longitudinal study design.

8.2.5.1. High frequency power.

Inclusion criteria for HF power were identical to those described in previously. Twenty potential studies into longitudinal changes in HRV were identified from the search criteria described previously. Of these, HF was not assessed in four studies (Reiling and Seals 1988; Seals and Chase 1989; De Meersman 1992; Levy *et al.* 1998). Additionally, HF data were recorded but not presented in one

study (Hautala *et al.* 2003). In a further study HF data were presented, but not in the required units (Portier *et al.* 2001). Finally, data were presented in a form that did not allow calculation of Cohen's *d* in two further studies. (Hedelin *et al.* 2000; Melanson and Freedson 2001).

Twelve studies including a total of 17 trials were entered into the analysis. Of these trials, two from one study (al-Ani *et al.* 1996) were rejected due, to very small ($n=2$) sample size and results deemed to be spurious. Briefly, an effect size four times the mean magnitude of all other results was reported. The direction of this effect was also in the unexpected direction one study group. The remaining subjects ($n = 7$) showed large changes in the expected direction despite exposure to the same training stimulus. This would seem to infer a methodological shortcoming in the data.

8.2.5.2. SDNN

Some studies included in the analysis of HF did not include measures of SDNN (Boutcher and Stein 1995; Perini *et al.* 2002; Uusitalo *et al.* 2002). Two studies were excluded from the analysis as SDNN was reported in a format that did not allow calculation of Cohen's *d* (Seals and Chase 1989; Melanson and Freedson 2001). Eleven trials were finally entered into the analysis for longitudinal changes in SDNN.

8.2.5.3. RR interval

Of the twelve studies entered into the previous analyses, RR data were not presented in one study (Hedelin *et al.* 2000). In two studies, RR interval data were not supplied in a way which facilitated the calculation of effect size (Melanson and Freedson 2001; Hautala *et al.* 2003). One study, identified as providing information on RR interval data over the course of an exercise intervention (Portier *et al.* 2001) was subsequently omitted from the analysis.

This was because neither HF nor SDNN were supplied in required units or in a suitable format for meta-analysis.

8.3. Statistical Methods.

To compare cross-sectional differences in HRV measures the effect size was calculated using Cohen's d statistic. Where possible this was done using means and standard deviations of the groups using the following formulae:

$$d = M^1 - M^2 / \sigma_{pooled} \quad \text{Equation 8-1.}$$

where

$$\sigma_{pooled} = \sigma_1^2 + \sigma_2^2 / 2$$

The same calculations were made when longitudinal designs were analysed. The pooled standard deviation was calculated from the pre- and post-test standard deviations of the experimental group. Previous studies (Davy *et al.* 1996; Hedelin *et al.* 2000) have suggested that HRV data does not demonstrate regression toward the mean following exercise intervention therefore allowing this choice of standard deviations to be used. This method of calculating σ may overestimate d slightly as it does not take into account the correlation between the two sets of scores (Dunlop *et al.* 1996). It was chosen because HRV is a dynamic measure which commonly shows variation from test-retest and large within group variation. These factors serve to reduce estimates of effect size. The use of this liberal method of effect size estimation was deemed to be advantageous as it allowed inclusion of studies where no control group was used.

8.3.1. Tests for Heterogeneity.

Heterogeneity between study results creates problems in meta-analysis (Higgins *et al.* 2002) and a number of sources are available providing guidance for dealing with this issue (Cook *et al.* 1995; Deeks *et al.* 1996; Clarke and Oxman 2000; NHMRC 2000). Recently, Higgins *et al.* (2002) reviewed these sources, synthesised their recommendations and created guidelines on how heterogeneity

may be best assessed and dealt with in meta-analysis. What follows is a brief summary of their findings and recommendations. All sources of guidance recommended the use of a statistical test for heterogeneity based either on the summary statistic (Q) of each study or the $Q/(k-1)$ statistic where Q is the χ^2 value in a test for heterogeneity and k is the number of studies in the meta-analysis. This statistic has the particular feature by which a value greater than one may be used to provoke further investigation even if the Q statistic itself is non-significant. Such a facility is helpful, as it means the statistic is independent of sample size.

Higgins *et al.* (2002) found that the various sources provided consistent advice about what to do is if heterogeneity was detected. All sources advocated that a random effects model be used either alongside or instead of a fixed effects model to account for unexplained heterogeneity. The Cochrane group provided specific guidelines for meta-analyses based on P -values of heterogeneity statistics. They stated that: if $P > 0.10$ a fixed effects model should be used, if $P = 0.05-0.10$ a random effects model should be employed and that if $P < 0.05$, no meta-analysis be performed.

Higgins *et al.* (2002) also advocated that a cautious examination of potential sources of heterogeneity be used. They found that all reviewed sources highlighted the possibility of spurious findings arising from subgroup analyses. The authors adopted previous recommendations (Oxman and Guyatt 1992) which state that a small number of analyses be undertaken on subgroups which have been determined *a priori*. Additionally, the selection of subgroups should be based on the following:

- i. causal mechanisms
- ii. magnitude of effects
- iii. statistical significance.

Higgins *et al.* (2002) made the additional recommendation that all subgroup analyses be physiologically justified. All the sources reviewed advocated caution in interpretation of subgroup analysis results. The decision tree below

(Figure 8-1) was used in the present study to check for heterogeneity and identify its causes.

The within-group heterogeneity was assessed by calculation of the Q statistic for all data in the analysis using the following formula.

$$Q = \sum w_i (\Theta_i - \Theta)^2 \quad \text{Equation 8-2.}$$

The squared distance of each study from the combined effect is calculated. Each value is weighted using the formula below, with a greater weight given to more precise studies. The 'se' is based on the inverse variance method and all calculations are based on log values (Borenstein and Rothstein 1999).

$$w_i = 1/[se(\Theta_i)] \quad \text{Equation 8-3}$$

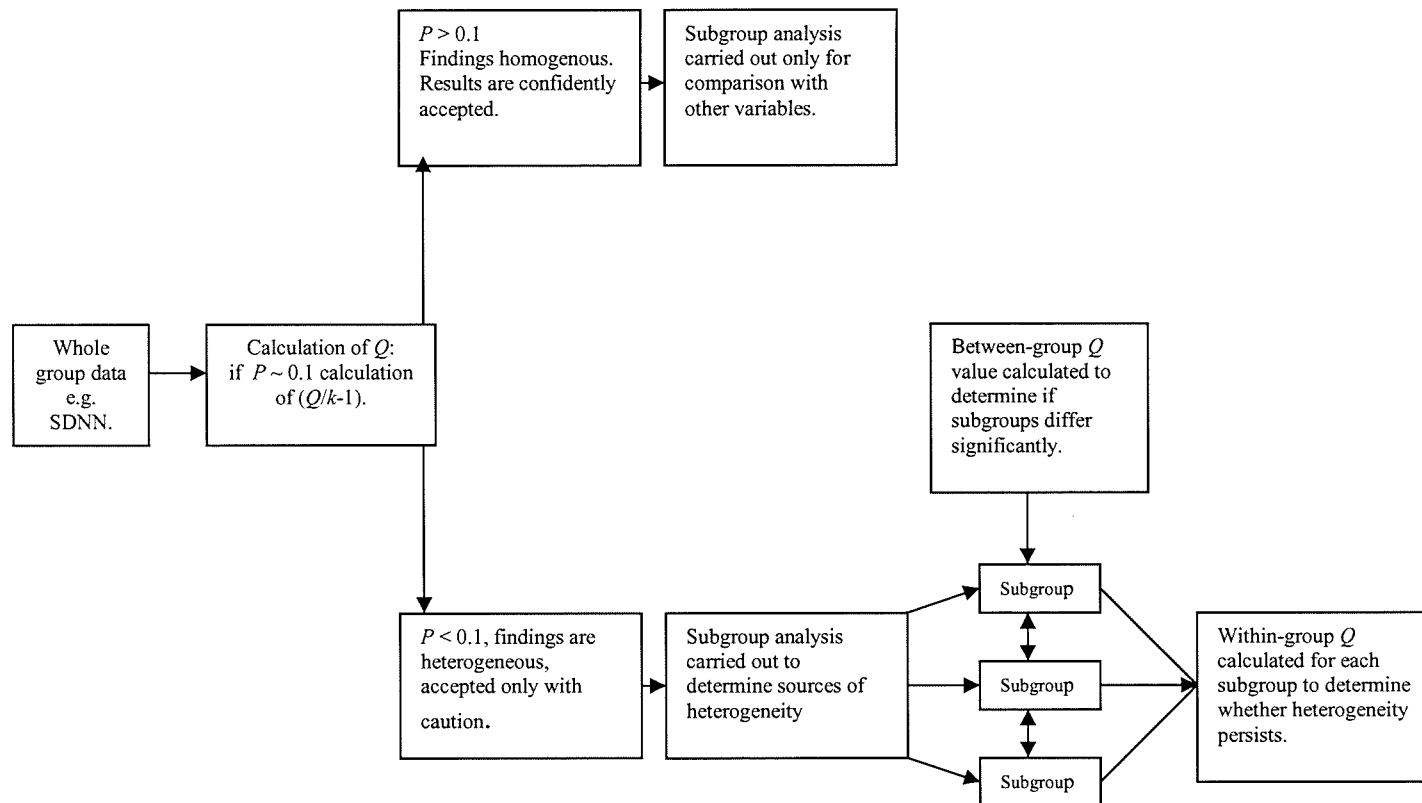


Figure 8-1 Process for the determination of effect size heterogeneity by subgroup analysis.

It should be noted that the level of significance employed when using the Q statistic is $P < 0.10$. This is due to the inherent weakness of the statistic in its ability to identify heterogeneity both within and between groups. Values which approached significance at $P = 0.10$ and those between $P = 0.10$ and 0.05 were investigated further using the $Q/(k-1)$ statistic.

8.3.2. *Subgroup comparisons.*

In keeping with current recommendations, *a priori* decisions regarding all subgroup analyses were made. In the cross-sectional meta-analyses only two subgroups were investigated. In the longitudinal analysis five subgroups were identified and investigated. The reasons for this larger number were because greater inference can be drawn from this type of analysis and because more possible moderator variables were evident in the literature.

8.3.2.1. Analysis of moderator variables in cross-sectional data.

From reviewing the data it was obvious that studies could be logically subdivided into two groups:

- i. Those that contrasted athletes (such as national standard runners or cross-country skiers) with sedentary controls.
- ii. Those that contrasted moderately 'trained' individuals (usually recreational athletes differentiated by higher $\dot{V}O_{2\max}$ or maximal work output) with sedentary controls.

Physiological justification for this subgroup allocation lies in the much greater differences in training (volume, intensity and duration) between groups in the 'athletes vs. controls' comparison and the 'trained vs. controls' comparisons. The preceding nomenclature will be used in the remainder of this chapter to identify these subgroups.

The second subgroup analysis was based on the two broad methodological categories of HRV data collection. These are either:

- i. Twenty four-hour, ambulatory monitoring of the ECG.
- ii. Resting, stable ECG recording made over short periods of time (commonly 2 – 20 min).

Although no direct physiological justification of these subgroups or prediction of effect direction can be given, the vast difference between these methodologies is clear and their analysis can be logically justified. In addition to this there is debate over the relative merits of each method and complex issues surrounding their application to HRV analysis (Camm *et al.* 1996) that are beyond the scope of this chapter.

8.3.2.2. Analysis of moderator variables in longitudinal data.

The potential moderator variables identified in this analysis were classified as either: subject characteristics, training intervention characteristics or HRV methodological characteristics.

The first classification of subgroup was subject characteristics. The first subgroup analysis in this group was based on subject sex. Trials including males, females and mixed groups are all present in the literature. Physiological justification for this subgrouping lies in the known differences in HRV measures between the sexes (Gregoire *et al.* 1996; Kuo *et al.* 1999) and the between-sex differences in HRV response to training which exist in the literature (Myslivecek *et al.* 2002).

The second subgroup analysis concerned subject age. Subjects were divided into three groups defined as young (mean age < 30 years) middle aged (mean age 30 - 60 years) or old (mean age > 60 years). This analysis was justified physiologically as differences in HRV are evident between age groups (Byrne *et al.* 1996; Kuo *et al.* 1999). It is also known that responses of other physiological measures to training are affected by age. The final subject characteristic use to define subgroups was previous training status. It is common for subjects with a

relatively high value for a given physiological characteristic to show less adaptation than those with lower initial values and *vice versa*. This phenomenon is known as law of initial values and statistically, it commonly translates into regression toward the mean. There is some evidence to suggest an opposite direction of effect for certain characteristics of HRV (Hautala *et al.* 2003). There are also data to suggest that high levels of HRV may prevent further increases in very fit subjects due to physiological factors such as acetylcholine saturation of the SA node (Goldberger *et al.* 1994; Eckberg 1997; Goldberger *et al.* 2001).

The second subgroup classification was differences of training intervention. However, the only analysis it was possible to carry out was based on intervention duration. The two subgroups analysed were comprised of studies ≤ 12 weeks duration and those >12 weeks. This point of division was derived from the literature as 12 weeks seemed to be an upper limit to the shorter interventions used. Above this point studies varied from 14 weeks to 2 years in duration. This was justified by the occurrence of such a wide range of intervention durations in the literature. Many physiological variables show differences in magnitude of adaptation dependent on duration of stimulus. It was felt, logically, that studies of 8-week duration could not be compared fairly with those of 1 year.

The last subgroup classification was HRV methodology and contained subgroup analysis between short-term and 24-hour ECG recordings as defined in section 8.3.2.1.

8.4. Results.

8.4.1. Heterogeneity of cross-sectional comparisons.

There was significant heterogeneity of results for differences in RR interval ($Q = 42.51, P < 0.0001$). The resulting value for $Q/(k-1)$ was 3.35. Similarly, there was significant heterogeneity of results for differences in RR interval ($Q = 37.40, P = 0.0001$) and the resulting value for $Q/(k-1)$ was 3.35. The results of

trials reporting differences in HF were also heterogeneous ($Q = 40.24, P = 0.0001$) with a resulting value for $Q/(k-1)$ of 3.40.

8.4.2. *Differences in RR-interval.*

Seventeen trials were drawn from the 14 studies that met the inclusion criteria (Figure 8-2). In total, 476 cases were included in this first part of the meta-analysis. The overall effect size was $d = 1.30$ (C.I. 0.61 – 1.99, $P = 0.0002$). When subgroup analysis between comparisons of athletes vs. controls or trained individuals vs. controls was carried out, the effect sizes were $d = 1.46$ (C.I. 0.44 – 1.60, $P = 0.0009$) and $d = 0.78$ (C.I. 0.32 – 1.24, $P = 0.001$) respectively. The trials comparing groups of athletes and controls included some mixed and anaerobically trained subject groups in addition to those who were solely aerobically trained. When such groups were removed, the overall effect size was increased to $d = 1.84$ C.I. (0.80 – 2.8, $P = 0.0002$).

Subgroup analysis revealed the mean effect size for athletes vs. controls to be significantly greater than that of trained individuals vs. controls when mixed and anaerobically trained groups were excluded ($Q = 4.31, P = 0.038$). This was not the case when ‘mixed training’ groups were included in the analysis ($Q = 1.73, P = 0.19$). Due to the observed effect of including such trials on overall effect size, these group comparisons were not entered into the remaining analysis for RR interval or any other HRV variables. Within-group heterogeneity was still evident in the athletes vs. controls group ($Q = 34.82, P = 0.0003$). The results of the trained individuals vs. controls group were found to be relatively homogenous ($Q = 3.4, P = 0.34$) although $Q/(k-1) = 1.7$.

When studies were compared by HRV measurement method there was a significant between groups effect ($Q = 6.94, P = 0.008$). Those studies using 24-hour measures showed a greater effect size ($d = 0.84, P = 0.004$) than those using short-term measures ($d = 0.26, P = 0.02$). When trials were divided in this way, results from those using short-term recordings remained heterogeneous

($Q = 32.01, P = 0.0009$) whereas results from 24-hour monitoring were homogenous ($Q = 1.28, P = 0.73$).

8.4.2.1. Differences in SDNN.

Twelve studies were entered into the meta-analysis with a total of 356 cases (Figure 8-3). The overall effect size for differences in SDNN was $d = 0.79$ (C.I. 0.40 – 1.18, $P = 0.0001$). When data were grouped by comparison, the effect size for athletes vs. controls ($n = 322$ cases) was $d = 0.81$, (C.I. 0.35 – 1.27, $P = 0.0006$). When trained subjects were compared with controls ($n = 43$ cases) the mean effect size was $d = 0.66$ (C.I. 0.04 – 1.27, $P = 0.0384$). The difference between these effect sizes was not significant ($Q = 0.002, P = 0.96$). Further calculations of within group Q -values revealed the athletes vs. controls group to still have heterogeneous effect sizes ($Q = 36.95, P = 0.00003$) whereas comparisons between trained individuals and controls were homogenous ($Q = 0.48, P = 0.48$).

When between-group differences based on data recording type were calculated the effect size gained from 24-hour recordings ($d = 1.00$ C.I. 0.58 – 1.46) was significantly greater ($Q = 3.86, P = 0.049$) than that for short-term measures ($d = 0.54$ C.I. 0.33 – 0.80). Assessment of within group heterogeneity following this subgroup analysis showed results from short-term recordings remained heterogeneous ($Q = 33.32, P = 0.0002$). Conversely, long term recordings showed homogenous results ($Q = 0.25, P = 0.96$).

8.4.2.2. Differences in HF

Thirteen studies with a total of $n = 422$ cases were entered into the final analysis for HF (Figure 8-4). Nine of these compared athletes and controls ($n = 327$ cases) the remainder compared trained individuals with controls ($n = 95$). Analysis of the HF data as a whole indicated a significant overall effect size of $d = 0.52$ (CI 0.14 – 0.88, $P = 0.006$).

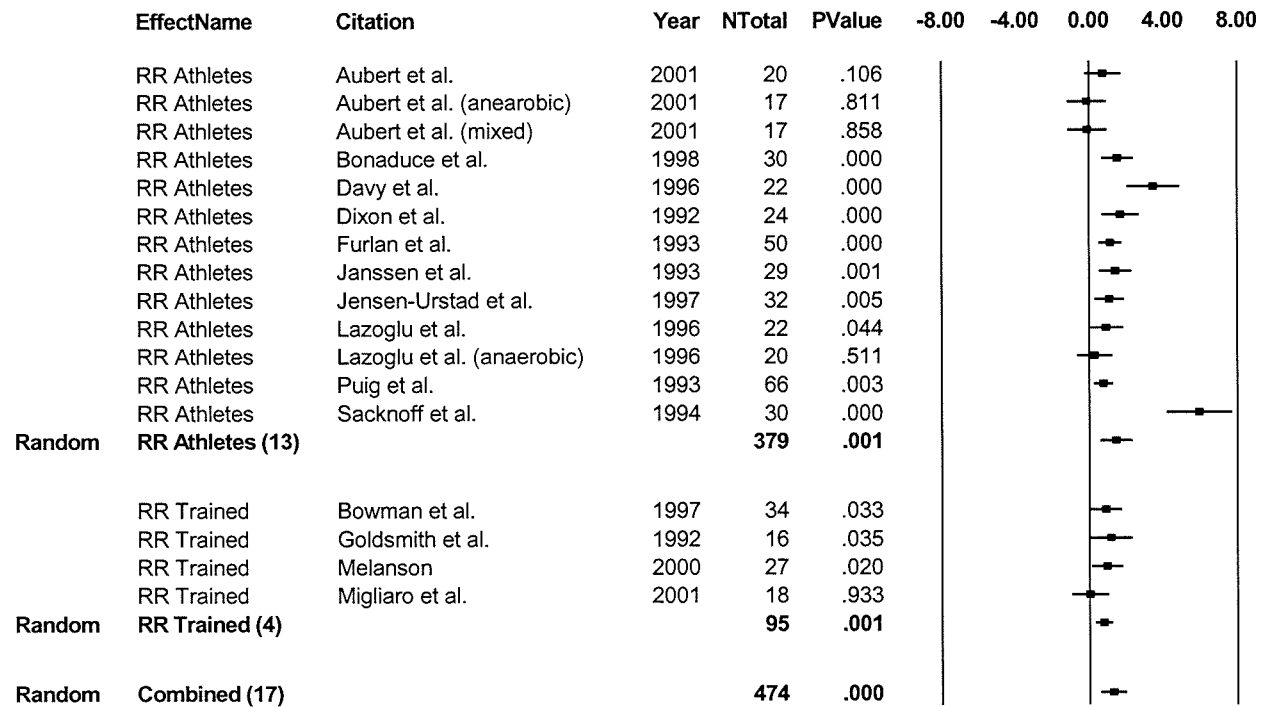


Figure 8-2 Cross-sectional comparison of RR interval between trained subjects/athletes and inactive controls.

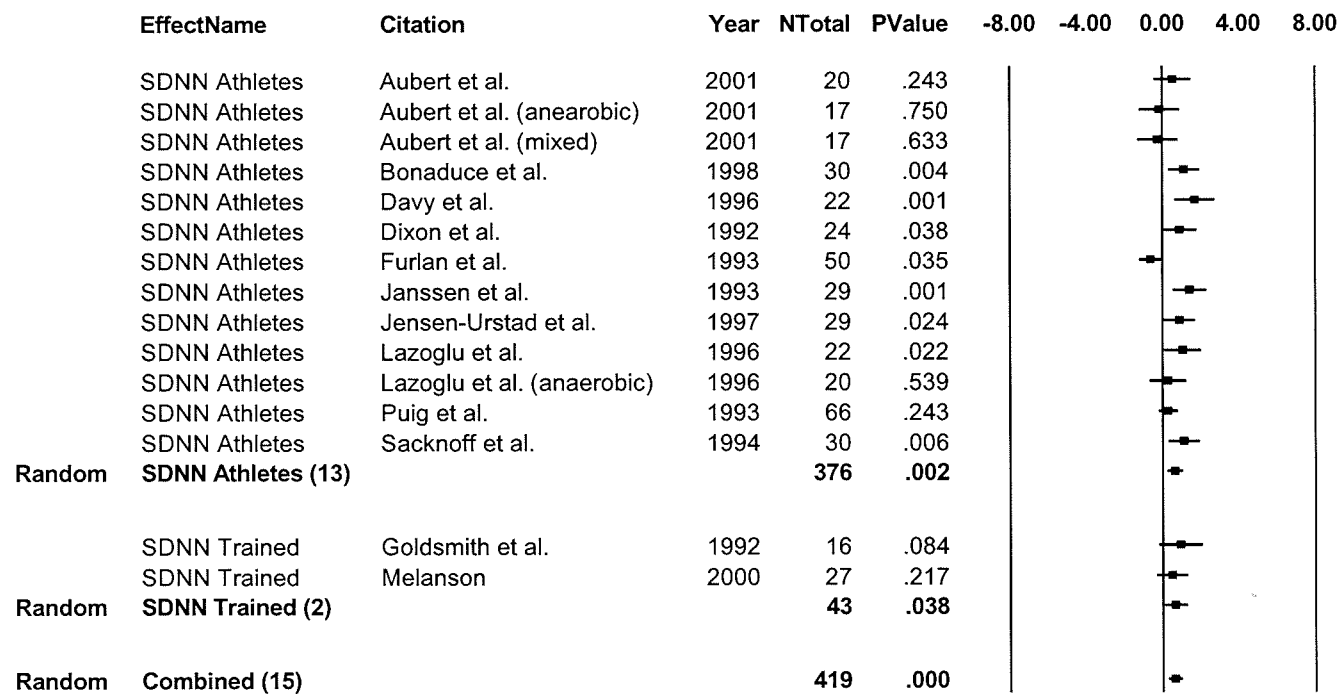


Figure 8-3 Cross-sectional comparison of SDNN between trained subjects/athletes and inactive controls.

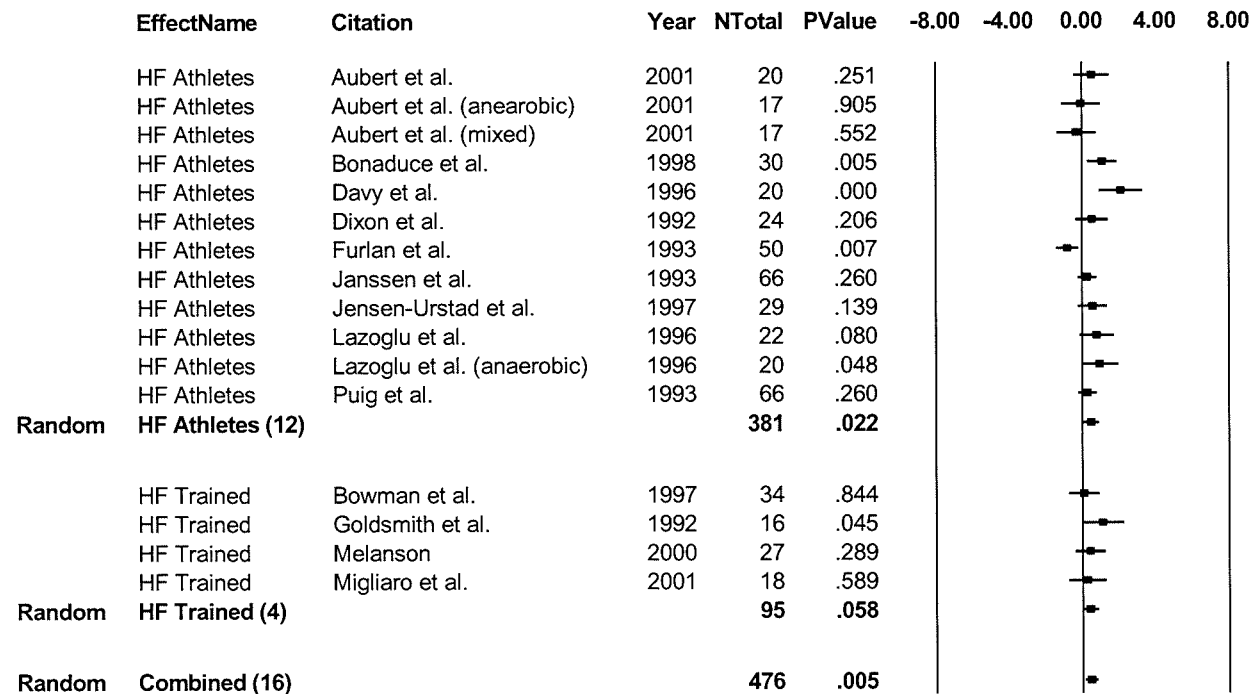


Figure 8-4. Cross-sectional comparison of HF power between trained subjects/athletes and inactive controls.

Subgroup analysis revealed the mean effect size for athletes vs. controls ($d = 0.55$, C.I. $0.07 - 1.04$, $P = 0.03$) was greater than that for trained individuals vs. controls ($d = 0.42$ C.I. $= -0.01 - 0.87$, $P = 0.058$) although this difference was not statistically significant ($Q = 0.02$, $P = 0.94$). Following this subgroup analysis, results from comparisons of athletes vs. controls remained significantly heterogeneous ($Q = 40.24$, $P = 0.0003$) whereas the results from trained individuals vs. controls were homogenous ($Q = 2.67$, $P = 0.45$).

When trials were grouped by data recording type the mean effect size for short-term recordings ($d = 0.42$, C.I. $0.36-0.48$) was significantly ($Q = 6.95$, $P = 0.008$) smaller than that found using 24-hour recordings ($d = 0.87$ C.I. $0.47 - 1.28$). Results of trials using short-term recordings remained heterogeneous ($Q = 32.01$ $P = 0.00009$) whereas results from long-term recordings were homogenous ($Q = 1.29$, $P = 0.73$)

8.4.3. *Longitudinal comparisons.*

8.4.3.1. Longitudinal changes in RR interval.

Figure 8-5 shows the results of the meta-analysis for changes in RR interval due to exercise training. Sixteen groups were drawn from 11 studies giving a total of 263 cases. The overall effect size was $d = 0.72$ (C.I. $0.24 - 1.19$, $P = 0.003$). However, the effect sizes for differences in RR interval were found to be highly heterogeneous ($Q = 50.42$, $P = 0.00001$, $Q/(k-1) = 3.45$).

8.4.3.2. Effect of moderator variables on changes in RR.

The moderator variable subgroup analysis (Table 8-1) revealed significant between-group heterogeneity for study subgroups based on subject age. Subject sex, length of exercise intervention and HRV data collection technique also showed between-group heterogeneity ($Q/(k-1) > 1$) and although values for Q were small they were statistically significant ($P = 0.09 - 0.10$). Subject activity

level prior to exercise intervention was also found to influence change in RR interval (Table 8-1) although no significant heterogeneity of effect sizes was found. The largest effect size in this analysis was for the previously sedentary subjects. This effect size remained highly heterogeneous after the data were subdivided into the two subgroups ($Q = 46.80$, $P = 0.0001$). This was also the case for intervention duration and HRV data collection subgroup analyses.

Table 8-1 The effect of moderator variables on effects sizes for changes in RR interval.

Effect name	Categories	Effect size (mean and 95%CI)	N total	P-value	Between-group Q-value	P-value	Within group Q-value	P-value
Sex	Female	0.747 0.03 – 1.45	43	0.278			2.1	$P=0.71$
	Male	0.799 0.44 – 1.15	166	0.001			4.6	$P=0.70$
	Mixed	0.39 0.15 – 0.93	54	0.173			38.5	$P<0.0001$
	Combined	0.715 ± 0.240	263	0.003	Q=4.50	$P=0.10$		
Age	Young	1.292 ± 0.870	51	0.144			Q=36.8	$P<0.0001$
	Middle aged	0.692 ± 0.163	151	<0.001			Q=2.8	$P=0.83$
	Old	0.193 ± 0.256	61	0.453			Q=1.3	$P=0.70$
	Combined	0.715 ± 0.240	263	0.003	Q=8.81	$P=0.01$		
Activity level	Active	0.436 ± 0.278	152	0.123			Q=1.8	$P<0.88$
	Sedentary	0.849 ± 0.331	111	0.011			Q=46.8	$P<0.0001$
	Combined	0.715 ± 0.240	263	0.003	Q=1.10	$P=0.29$		
Intervention duration	Long duration	1.06 ± 0.413	181	0.185			Q=42.0	$P<0.0001$
	Short duration	0.396 ± 0.221	82	0.001			Q=4.23	$P=0.83$
	Combined	0.715 ± 0.240	263	0.001	Q=2.72	$P=0.09$		
HRV recording	24-hour ambulatory	0.349 ± 0.238	71	0.146			Q=2.01	$P=0.57$
	Short-term recording	0.829 ± 0.320	192	0.010			Q=45.6	$P<0.0001$
	Combined	0.703 ± 0.241	263	0.004	Q=2.82	$P=0.09$		

8.4.3.3. Changes in SDNN.

Figure 8-6 shows the results of the meta-analysis for changes in SDNN interval due to exercise training. Seventeen trials were drawn from 12 studies giving a total of 230 cases. The overall effect size was $d = 0.827$ (C.I. 0.36 – 1.30, $P = 0.001$). The effect sizes for differences in SDNN were found to be highly heterogeneous ($Q = 37.72$, $P = 0.0006$, $Q/(k-1) = 2.35$).

Table 8-2 The effect of moderator variables on effects sizes for changes in SDNN

Effect name	Categories	Effect size (mean \pm SEM)	N total	P-value	Between group Q value	P-value	Subgroup Q value	P-value
Sex	Female	0.573 \pm 0.428	43	0.190			Q=4.90	$P=0.18$
	Male	1.035 \pm 0.410	128	0.013			Q=0.70	$P=0.71$
	Mixed	0.393 \pm 0.270	55	0.152			Q=30.4	$P=0.00008$
	Overall	0.781 \pm 0.243	226	0.002	Q=1.66	$P = 0.44$		
Age	Young	0.833 \pm 0.358	93	0.023			Q=4.1	$P=0.19$
	Middle aged	0.422 \pm 0.207	61	0.045			Q=20.51	$P=0.00004$
	Old	0.231 \pm 0.256	63	0.372			Q=9.67	$P=0.08$
	Overall	0.497 \pm 0.153	217	0.001	Q=2.36	$P = 0.31$		
Activity level	Active	0.721 \pm 0.274	64	0.011			Q=7.04	$P=0.31$
	Sedentary	0.253 \pm 0.168	142	0.134			Q=1.03	$P=0.95$
	Combined	0.388 \pm 0.139	206	0.006	Q=2.1	$P = 0.15$		
Intervention duration	Long duration	0.478 \pm 0.230	71	0.204			Q=29.83	$P<0.00001$
	Short duration	0.523 \pm 0.257	146	0.004			Q=7.8	$P=0.25$
	Combined	0.489 \pm 0.165	217	0.001	Q=0.08	$P = 0.89$		
HRV recording	24-hour ambulatory	0.305 \pm 0.204	71	0.204			Q=1.05	$P=0.79$
	Short-term recording	0.621 \pm 0.211	146	0.004			Q=13.6	$P=0.13$
	Overall	0.497 \pm 0.153	217	0.001	Q=0.71	$P = 0.40$		

8.4.3.4. The effect of moderator variables on longitudinal changes in SDNN.

The effect of previously selected moderator variables for values of d , representing changes in SDNN are shown in table 8-2. None of the moderator variables showed statistically significant differences between groups. The largest between-group variation was found for activity level. Although not statistically significant ($Q = 2.1, P = 0.15$) the effect sizes for active individuals were more than twice that of sedentary counterparts indicating this result to be somewhat heterogeneous ($Q/(k-1) = 1.05$).

Similarly, other moderator variables clearly influenced effect size. Visual analysis of table 8-2 shows sex (females) age (young) and HRV recording (short-term) all to have effect sizes approximately double that of other subgroups. None of these overall effects were statistically significant and large within-group heterogeneity still existed in certain subgroups following these analyses.

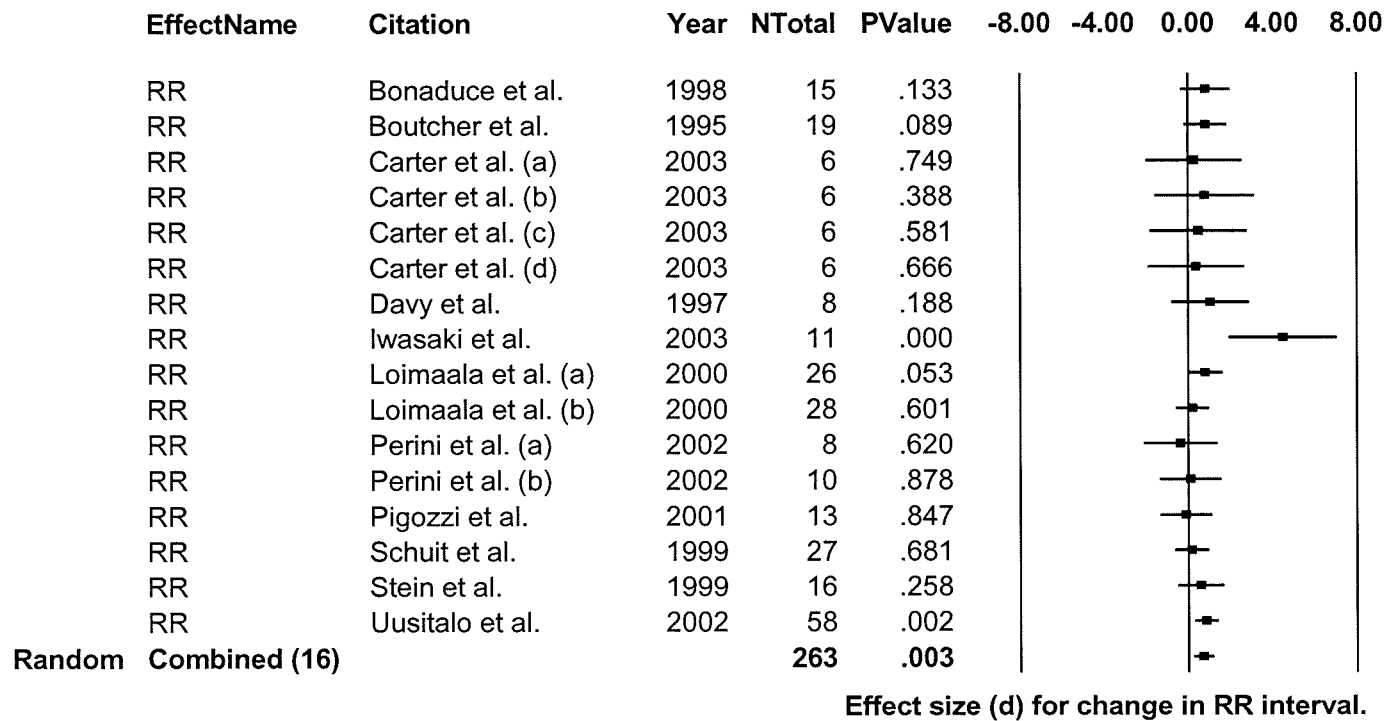


Figure 8-5. Longitudinal analysis of change in RR interval due to exercise training.

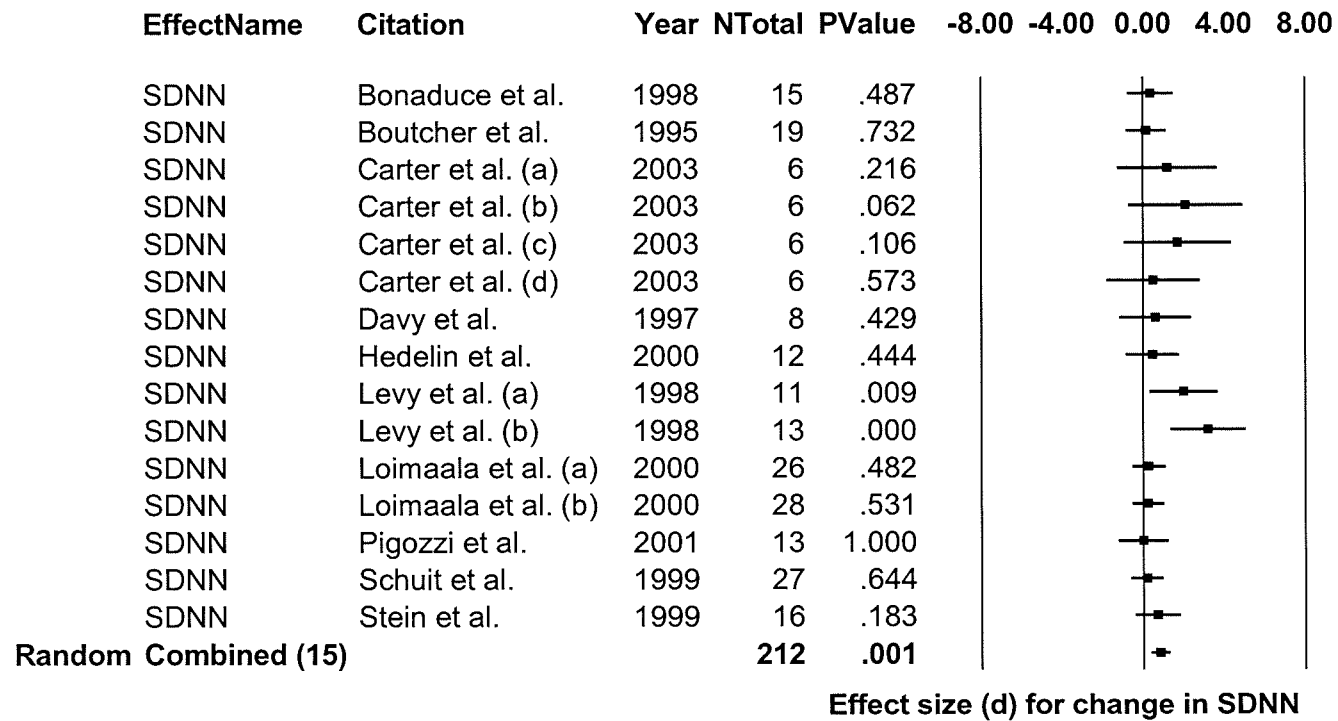


Figure 8-6. Longitudinal analysis of change in SDNN due to exercise training.

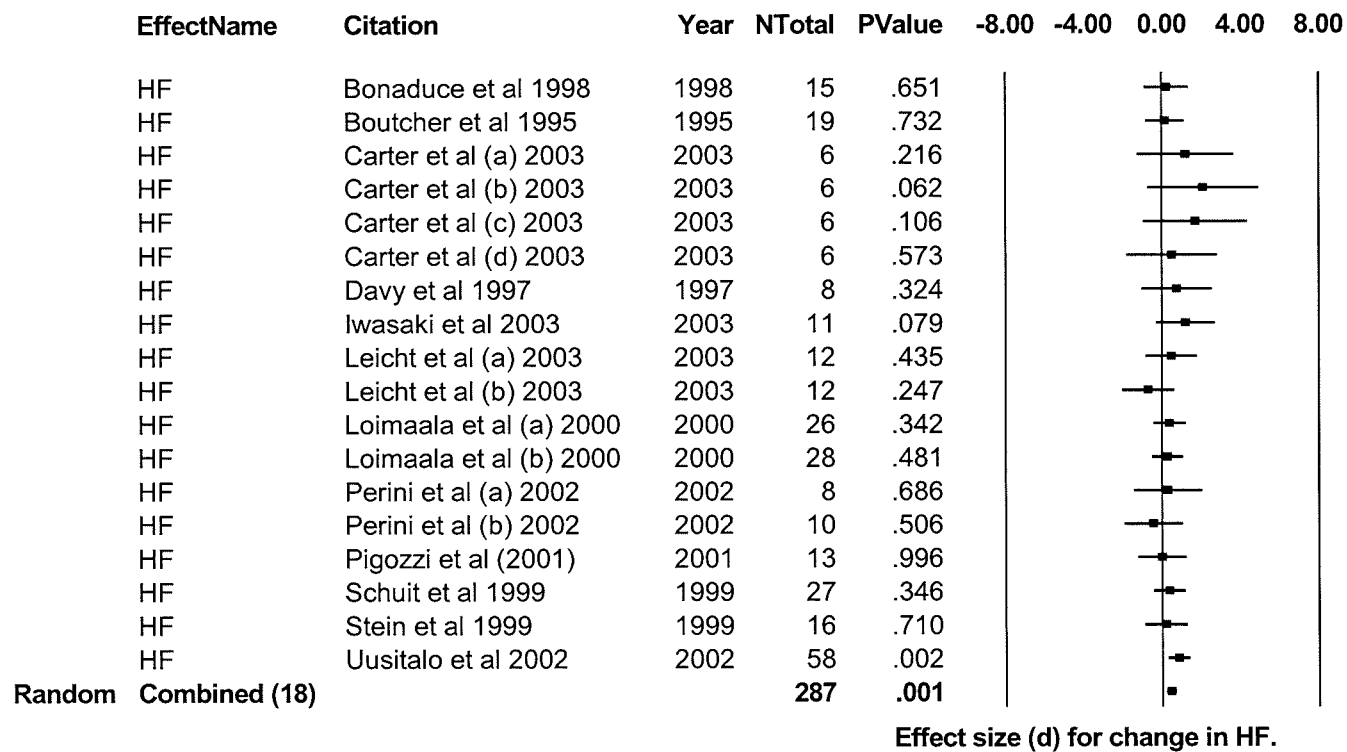


Figure 8-7. Longitudinal analysis of change in HF power due to exercise training.

8.4.3.5. Longitudinal changes in HF.

A total of 18 group comparisons from 12 studies were entered into the final analysis. A total of 287 cases were analysed and the overall effect size was $d = 0.45$ (CI 0.20 – 0.71, $P = 0.001$). The effect sizes for differences in HF were found to show statistical homogeneity ($Q = 19.16$, $P = 0.32$) although limited evidence of heterogeneity was evident ($Q/(k-1) = 1.12$).

Table 8-3. The effect of moderator variables on effects sizes for changes in HF power.

Moderator variable	Subgroup	Effect size (mean \pm 95% CI)	N total	P-value	Between group Q	P-value	Subgroup Q	P-value
Sex	Female	0.605 (0.1 – 0.9)	43	0.168			Q=6.57	$P=0.09$
	Male	0.536 \pm 0.410	166	0.001			Q=4.86	$P=0.56$
	Mixed	0.155 \pm 0.297	78	0.273			Q=5.36	$P=0.06$
	Combined	0.454 \pm 0.129	287	0.001	Q=0.76	$P = 0.68$		
Age	Young	0.457 \pm 0.372	63	0.223			Q=10.9	$P=0.50$
	Middle aged	0.618 \pm 0.163	151	0.001			Q=5.29	$P=0.83$
	Old	0.228 \pm 0.234	73	0.333			Q=1.44	$P=0.86$
	Combined	0.497 \pm 0.153	287	0.001	Q=2.02	$P = 0.36$		
Activity level	Active	0.413 \pm 0.131	52	0.025			Q=7.26	$P=0.20$
	Sedentary	0.454 \pm 0.129	235	0.002			Q=11.0	$P=0.44$
	Combined	0.388 \pm 0.139	287	0.001	Q=0.91	$P = 0.34$		
Intervention duration	Long duration	0.454 \pm 0.163	189	0.185			Q=7.95	$P=0.16$
	Short duration	0.541 \pm 0.259	82	0.001			Q=9.59	$P=0.21$
	Combined	0.469 \pm 0.137	271	0.001	Q=0.04	$P = 0.97$		
HRV recording	24-hour ambulatory	0.305 \pm 0.204	71	0.204			Q=0.31	$P=0.96$
	Short-term recording	0.621 \pm 0.211	146	0.004			Q=17.6	$P=0.17$
	Combined	0.497 \pm 0.153	217	0.001	Q=1.27	$P=0.26$		

8.4.3.6. Effects of moderator variables on longitudinal changes in HF.

Subgroup analysis revealed no statistically significant between-group heterogeneity. However, between age subgroups, group containing older subjects clearly showed smaller overall effect sizes than either young or middle-aged groups. Additionally, there was a clear trend toward larger (double) effect sizes in trials which used short-term compared with long-term HRV data recordings (Table 8-3).

8.4.4. *Relationships between effect sizes for HRV variables.*

Table 8-4 shows the Pearson product moment correlation coefficients between each pair of variables from cross-sectional and longitudinal meta-analyses.

Table 8-4. Relationships between variables for all analyses.

Measures	Correlation coefficient	<i>P</i> – value (2-tailed)	Correlation coefficient	<i>P</i> – value (2-tailed)
	Cross-sectional comparisons		Longitudinal comparisons	
SDNN vs. RR	R = 0.727	<i>P</i> = 0.003	r = 0.346	<i>P</i> = 0.226
HF vs. RR	R = 0.660	<i>P</i> = 0.005	r = 0.345	<i>P</i> = 0.191
SDNN vs. HF	R = 0.816	<i>P</i> = 0.0001	r = 0.971	<i>P</i> = 0.0001

For cross-sectional comparisons, changes in RR interval were significantly and positively correlated with changes in SDNN and HF. The relationship between effect sizes for SDNN and HF was also a very strong positive correlation. When effect sizes for longitudinal change in these three measures were correlated, effect sizes for change in HF and SDNN only correlated weakly with RR interval ($P > 0.05$), however, the linear relationship between SDNN and HF remained very strong ($P < 0.0001$)

Table 8-5. Effects of different fits on percentage variance explained between measures.

Relationship	Regression.	R^2	P - values
RR vs. SDNN	Linear	0.119	0.226
RR vs. SDNN	Quadratic	0.143	0.427
RR vs. SDNN	Cubic	0.262	0.365
RR vs. HF	Linear	0.119	0.191
RR vs. HF	Quadratic	0.174	0.289
RR vs. HF	Cubic	0.177	0.489
SDNN vs. HF	Linear	0.943	0.0001
SDNN vs. HF	Quadratic	0.945	0.0001
SDNN vs. HF	Cubic	0.946	0.0001

In addition to assessing linear relationships between RR interval change effect sizes and HRV effect sizes, a variety of fits were applied to the data to assess this relationship more fully. By a serial fitting process of curve estimation it was found that a linear relationship was not the best method by which to describe the relationship between RR interval and either SDNN or HF effect sizes (Table 8-5). The weak linear relationship between effect size for change in RR interval and SDNN was improved by fitting a cubic regression line to the data. Compared to a simple linear fit, the cubic regression could account for more than twice the variance between these variables. A slight improvement in the amount of shared variance accounted for between RR and HF was also achieved using a cubic fit. Neither of these fits achieved statistical significance. The variance accounted for between SDNN and HF was practically unchanged by the fitting of either a quadratic or cubic regression to the data.

8.5. Discussion.

Bradycardia is a commonly observed, chronic physiological response following exercise training (Raab *et al.* 1960; Frick *et al.* 1967; Ekblom *et al.* 1973; Scheuer and Tipton 1977). It is commonly believed that exercise-induced bradycardia is at least partially mediated by increased vagal tone at rest. There are much data to suggest that heart rate variability measures can be used as indices of vagal tone at rest in humans. The aims of this meta-analysis were therefore:

- i. To identify whether a resting bradycardia was accompanied by increased global and vagally mediated measures of HRV in cross-sectional comparisons.
- ii. To assess the effects of exercise interventions on resting bradycardia and HRV measures.
- iii. To assess homogeneity of results and where possible, identify moderator variables which accounted for any heterogeneity in the above analyses.
- iv. To describe and quantify any relationships between differences and changes in the variables analysed.

8.5.1. *Cross sectional analyses.*

The cross-sectional data included in the present meta-analysis clearly show resting bradycardia is accompanied by increased vagal tone as measured by global (SDNN) and vagal (HF) markers of HRV. All three measures demonstrated statistically significant heterogeneity of effect sizes ($P < 0.0001$). Therefore, although the overall effect sizes for differences in all these variables are large and significant ($d > 0.6$, $P < 0.001$) these results must be treated with caution. Despite the use of a random effects model, generalisation of the findings of the current analysis must be carried out with caution.

8.5.1.1. Effects of moderator variables on cross-sectional data.

In an attempt to explain the heterogeneity of effect sizes a small number of moderator variables were investigated using subgroup analysis. Decisions on the subgroup analyses were made *a priori* and only a small number of potential moderator variables were investigated in line with current recommendations (Jennings *et al.* 2002).

As would be expected, when aerobically trained athletes were compared with controls, the magnitude of difference in RR interval length was greater than when trained individuals (commonly recreational athletes) were compared to similar controls. Both athletes vs. controls and trained individual vs. controls subgroups showed statistically significant differences, demonstrating that bradycardia is also evident in recreational athletes. The mean effect size for this comparison was $d = 0.78$. However, the confidence intervals associated with this value (0.32 – 1.24) illustrate the heterogeneous nature of the data. This heterogeneity was further confounded by the small number of studies which were entered into the analysis ($n = 4$) which yielded a small overall number of cases ($n = 95$).

The subgroup analysis of effect sizes based on the HRV recording method demonstrated that a significant amount of the heterogeneity observed in RR interval differences could be accounted for by this moderator variable. Studies using 24-hour recordings had a significantly greater mean effect size than the smaller number of studies using short-term stationary measures. It is known that resting bradycardia is most evident during sleep. Athletes have been reported to have RR intervals >2 sec during night hours (Goldsmith 1992). Although only complete 24-hour recordings were entered into the analysis, this greater effect size may be due to the inclusion of nocturnal RR interval data. On this basis it may be argued that if researchers want to quantify the full extent of vagal modulation on RR interval then 24-hour recordings may be a preferred method. Alternately, there are several methodological and practical issues associated with

the use of such measures that make short-term measurements of stationary ECG signals more appealing. These are discussed in more detail in section 5.5.1.3

Both HRV measures demonstrated a similar pattern of effect sizes. The global HRV measure, SDNN (which does not strictly differentiate between vagal and sympathetic sources of variation in heart period) was significantly greater in athletes and trained groups than controls. In keeping with the pattern of effect sizes for RR interval, the differences were greater in the athletes vs. controls comparison, indicating that longer RR intervals in athletes were accompanied by greater HRV.

High frequency spectral power (HF) is known to be a specific vagal indicator (Akselrod *et al.* 1981; Pagani *et al.* 1986; Tulppo *et al.* 1996; Nakata *et al.* 1998; Uusitalo *et al.* 2002). Overall, cross-sectional studies showed HF to be significantly elevated ($P = 0.006$) in active people and athletes compared to controls. Interestingly the subgroup analysis effect sizes were similar in magnitude to those observed for RR interval. High frequency power was found to be elevated in athletes compared to controls and less so when trained individuals were compared to inactive controls. This latter comparison failed to reach statistical significance. This was due in part, to the smaller sample size ($n = 95$). However, a reduced effect size, ($d = 0.55$ vs. 0.42) was also evident.

As with the RR interval data, effect sizes for SDNN and HF were greater when extracted from 24-hour ECG recordings than when short-term recordings were used. This may be due, again, to the greatest expression of increased vagal tone commonly observed during sleep. This would seem to suggest that ambulatory measures are favourable to short-term ECGs to evaluate HRV. It should be made clear however, that none of the studies included in this analysis give details of the activity levels of subjects during Holter recording. This is one factor which has previously been reported to influence SDNN significantly (Bernardi 1996). Additionally, one assumption underlying the computation of HF spectral power is stationarity of the time series from which it is calculated. By the very nature

of the recording method, this assumption is commonly not met when ambulatory ECG recordings are used.

8.5.1.2. Correlations between effect sizes.

To quantify the influence of increased vagal modulation on RR interval, the common variance between the HRV measures and RR interval was calculated. Simple linear regression showed that SDNN and RR interval shared the most common variance (53%) and HF shared slightly less (43.5%). This is surprising as HF is regarded as a specific, vagal marker of ANS function. However, it should be noted that not all studies that included HF also reported SDNN and vice versa. Due to the relatively small number of studies included in the analysis, a single study such as Sacknoff *et al.* (1991), which demonstrated a very large effect size for RR interval and SDNN, can affect the strength of this relationship considerably. Interestingly, the highest shared variance in this portion of the analysis was between HF and SDNN in which 66.7% of variance was common to both measures. The relationship between effect sizes for HF and SDNN is similar to published values for individual subject scores of these variables (Bigger *et al.* 1992).

The data from analysis of cross-sectional studies clearly shows a distinct bradycardia in physically trained individuals, and that a dose-response may be evident. Differences in RR interval and HRV markers show similar patterns overall, and within subgroups, suggesting that increase in vagal modulation may responsible for at least some (around 50%) of the resting bradycardia observed. These data also suggest that the use of 24-hour ambulatory ECG recordings may be favourable if differences in RR intervals and HRV measures are to be found. Causal inferences cannot be made from cross-sectional data due to the unknown status of the subjects prior to training. Biological influences such as heredity and methodological influences such as self-selection mean that differences cannot be attributed solely to differences in physical activity levels. For this reason meta-analysis of longitudinal data concerning changes in RR interval and HRV measures were also carried out.

8.5.2. Longitudinal analysis.

Meta-analysis showed a significant effect of exercise training on resting RR interval. Although the large effect size was accompanied by relatively small confidence intervals (Table 8-1) the effect sizes of the studies included in the analysis were found to be significantly heterogeneous. Therefore, as with the findings of the cross-sectional analysis, these data must be interpreted and generalised with caution, despite the use of a random effects model.

Resting bradycardia is well documented in the literature (Raab *et al.* 1960; Frick *et al.* 1967; Ekblom *et al.* 1973; Scheuer and Tipton 1977) and the overall effect of exercise on RR interval strongly support the effect of exercise in promoting bradycardia ($d = 0.72$, C.I. 0.24 – 1.19, $P = 0.003$). However, the cause is a matter of some discussion (Katona and Jih 1975; Maciel *et al.* 1985). To elucidate the influence of increased vagal modulation on RR interval due to training, the HRV measures of SDNN and HF were again analysed.

The measure SDNN provides only a global index of HRV. This measure does not distinguish between vagally and sympathetically mediated variations in HF. However, at rest it is assumed that the vagal branch of the ANS is the dominant effector, and therefore, that SDNN is predominantly mediated by vagal modulation. Meta-analysis of SDNN showed a large, positive overall effect of exercise training on HRV. Effect size and confidence intervals were similar in magnitude to that reported for RR interval, providing some support for a relationship between changes in SDNN and RR interval. Similarly to RR interval, the effect sizes for trials assessing SDNN were highly heterogeneous. Again, generalisation and interpretation of these data should be employed only with caution.

Meta-analysis of trials reporting HF showed a moderate, positive effect size ($d = 0.45$) indicating an increase in vagally mediated rhythmic oscillations from 0.15-0.40 Hz. These data strongly support the notion that increased RR interval length is mediated by increased vagal modulation (Frick *et al.* 1967; Ekblom *et al.* 1973; Smith *et al.* 1989). Additionally, the effect sizes of trials reporting

changes in HF due to training were homogenous. On this basis, the evidence provided by the random effects model used with these data may be interpreted with confidence in showing that HF is increased by exercise training in a variety of subject populations.

8.5.2.1. Effects of moderator variables on longitudinal data.

Prior to discussion of the effects of moderator variables on changes in HR and HRV, it is first necessary to explain why a full meta-regression was not carried out on these data. The process of meta-regression facilitates the identification of variables which predict the outcome (in this case effect size) variable of a meta-analysis. Commonly these include variables concerning the study population such as subject age and sex, and variables concerning any intervention such as duration. By regressing continuous data for such variables against the outcome variables the relationships between dependent and independent variables can be assessed. However, in order to do this a degree of heterogeneity between the studies entered into the analysis is necessary. Furthermore, the majority of studies included must provide the necessary data to carry out the analysis.

In the present analysis, the studies included varied greatly in subject group, nature of the intervention used and the HRV data collection used. Additionally, numerous studies omitted several important covariates that would have created gaps in the analysis. This would have weakened the analysis further making any powerful regression statistic impossible due to the relatively small total number of studies originally included.

In order to gain insight into the effects of moderator variables a series of subgroup analyses were carried out to determine differences between effect sizes grouped by logical, predefined variables. These were classified as concerning either: subjects, intervention or methodology.

8.5.2.2. The effect of moderator variables on RR interval change.

Subject age had a significant effect on change in RR interval due to training. Middle-aged subjects showed the greatest increase in RR interval compared to

young and old subjects. Middle-aged subjects also made up the greatest proportion of the subjects studied. This may be because younger subjects typically demonstrate longer RR intervals than older counterparts and therefore little improvement is likely to be seen in these individuals. If this were the case however, one would expect older subjects to demonstrate the greatest increase.

A possible reason why this was not evident may be that exercise intensity in studies using older subjects is commonly of a lower absolute, and often relative exercise intensity. Alternatively, it may be that older subjects (who typically show low levels of activity) may have a compromised ability to adapt to a given exercise training stimulus when compared with younger counterparts. However, evidence from individual trials in which older and younger subgroups have been exposed to similar exercise stimuli (Leicht *et al.* 2003; Carter *et al.* 2003) do not support this notion.

Exercise intervention duration showed an unexpected effect that was close to statistical significance. It was found that greater changes in RR interval were found in short (12 weeks or less) interventions compared to those of longer duration. Commonly within the literature, shorter interventions use higher exercise intensities, although no statistical test of this trend was performed. Additionally, it may be that adaptations in the ANS, which cause the observed bradycardia, are effective by 12 weeks. This would mean the expression of such an effect would be evident at this time point. Empirical data from longitudinal analysis of RR interval during training support this (Melanson and Freedson 2001; Iwasaki *et al.* 2003).

Of methodological interest is the moderator variable HRV recording. This has been discussed briefly in relation to cross sectional data but warrants further discussion here. It is recommended that two broad categories of HRV measurements be made: ambulatory 24-hour recordings and resting measures of 2 – 7 min (Camm *et al.* 1996). Little data are available on which of these types of measurement are more sensitive. However, in the present study the effect size for change in RR interval was greater when derived from short-term recordings than from 24-hour data. This is in direct conflict with the data from cross-sectional

comparisons. These conflicting findings cannot be explained at this time. What is of note is the highly significant within group heterogeneity ($Q = 45.6$, $P < 0.00001$) that is evident in the short-term recording subgroup, which warrants further investigation.

Due to the strong influence of this methodological factor on effect size, it would have been preferable to conduct a further subgroup analysis for all variables recorded by each method separately. However, this was deemed to be intractable due to small group numbers within the subgroups, especially the 24-hour group ($n = 4$ studies, $n = 71$ cases).

8.5.2.3. Effects of moderator variables on SDNN.

The largest between-groups effect on SDNN was due to age. Effect sizes for young subjects were on average twice that of middle aged and four times that of old subjects. These findings agree with data from an individual study in which age group comparisons were made (Carter *et al.* 2003). These researchers found significant increases for SDNN in young males but not in their older counterparts. In females no significant findings were evident but the effect size was larger in younger subjects. The non-significance of these findings are due in part to the small sample size used. The congruence of findings from the present analysis and those of Carter *et al.* (2003) demonstrate the usefulness of the meta-analysis procedure.

A non-significant, difference in mean effect size was evident between groups based on subject sex. Females demonstrated an effect size twice that of males. Similar to the findings for younger subjects in the age subgroup analysis, values for SDNN are known to be higher in females when compared to males at all ages (Kuo *et al.* 1999). The pattern of change denoted here by the effect sizes within subgroups is similar to one previously noted for HF power (Hautala *et al.* 2003). This pattern was, however, not evident within the HF data in the present study as these data yielded homogenous results. However, as the majority of data that made up the analysis were from resting (supine) stationary tachograms, it can be argued that HF and SDNN should both be measuring predominantly vagal

modulation of HR. On this basis SDNN may be seen as analogous to HF and therefore, to respond in a similar manner. Hautala *et al.* (2003) have shown that subjects with higher initial values of HRV (HF) also show greater increases in these measures compared to subjects with lower initial values in response to training. An association between HF and other adaptations to training has also been demonstrated (Hautala *et al.* 2003) and vagally mediated HRV has therefore been proposed as a possible marker of systemic and local plasticity or trainability. Again, this phenomenon warrants further investigation.

8.5.2.4. Effect of moderator variables on HF.

As the whole group meta-analysis was homogenous for HF effect sizes, a subgroup analysis was not necessary. It was, however, still carried out in order to gain insight into possible reasons for heterogeneity in the other measures analysed in the review. As would be expected, no significant effects of moderator variables were found in the analysis of HF. Similarly to the patterns shown for RR interval and SDNN there was a trend towards larger effect sizes for changes in HF in younger and middle aged subjects when compared with older counterparts. The reduced HF adaptation to a given training stimuli in older subjects may be of importance if increasing HF (vagal modulation) is to be put forward as a potential beneficial outcome of physical training or rehabilitation

A further evident pattern was that of larger effect sizes reported for short-duration measurements compared with 24-hour data. This difference was, again, similar in magnitude to those reported for RR interval and SDNN. It may be taken as evidence to suggest that when observing intrasubject changes in HR and HRV, that short-term measurement of a resting ECG is a method which possesses superior sensitivity to 24-hour ambulatory measures. Regardless of whether this is true, it can certainly be argued that, particularly for spectral analysis, this method is superior in terms of the underlying assumptions behind the analysis being met. Additionally, the ease by which such a measure may now be made and analysed makes it far superior in terms of accessibility and economy when compared with ambulatory ECG measurement.

8.5.2.5. Problems with subgroups analysis.

Despite large differences in mean effect sizes for subgroup analyses of all three measures there were few statistically significant findings. One reason for this was the large within group heterogeneity of effect sizes that remained in almost all subgroups. The reasons for such heterogeneity may be fourfold. Firstly, the relatively small number of trails and cases in the overall analysis naturally translate into small numbers in certain subgroups. This, in turn, means that small disparities in direction and magnitude of effects give rise to large confidence intervals for a given overall effect size and therefore create significant heterogeneity.

Secondly, it may be that the varied empirical methods employed in studies are responsible. In compiling this analysis a large number of studies were reviewed and rejected. Yet, a number of studies are included in this analysis that perhaps would not be entered if it were possible to gain sufficient case numbers using more strict criteria. For example many studies do not include a control group. This is a commonly used criteria for inclusion in meta-analyses in other areas, but in the present situation the number of cases included would be too small to facilitate any analysis.

A third reason may be that the moderator variables responsible for the differences in effect sizes between groups were not identified. Evidence for this lies within the large with-group heterogeneity that was commonly preserved following subgroup analysis. However, in any meta-analysis a balance between the number of subgroup analyses made and finding the reasons for spreads of values must be met. The subgroups analysed in the present study were numerous in comparison with other analyses. They were however, made *a priori* on the basis of physiological justification in line with current recommendations (Jennings *et al.* 2002).

The final reason for the heterogeneity of results is that this may be a feature of HRV measurement. Studies measuring changes in HRV commonly show very disparate effect sizes. For instance the data of al-Ani *et al.* (1992) met the

criteria for inclusion in this analysis but after initial entry were removed due to their disparate nature. In this study, all subjects were exposed to a similar training stimulus. Despite this changes in HRV were found to differ both in magnitude and direction between subjects. In addition to this it is commonly found that baseline values for HRV measurements also show very large between-group variation. It is therefore unsurprising that large differences in effect sizes for change in such variables are commonplace.

8.5.3. *Correlations between effect sizes.*

Although RR interval was positively related to SDNN and HF change, this association was only weak, explaining less than 12% of the variance between measures (Table 8-4). Effect sizes for SDNN and HF were very highly correlated and it therefore seems suitable to suggest that these two variables are measuring similar aspects of HRV. Further to this, the notion that at rest, variation in HR is predominantly under vagal control is also supported.

Visual analysis of these data suggested a simple linear relationship was not the best method to describe the relationship between effect sizes for HRV measures and RR interval. By fitting serial plots to these data it was possible to explain a greater proportion of the shared variance between SDNN and RR. Using a cubic model significantly improved the description of this relationship, accounting for 26% of the common variance (Table 8-5). The effects of different fits on the relationship between HF and RR were broadly similar in direction but of slightly lesser magnitudes. Again a cubic model gave a better description of this relationship but only managed to explain a small additional amount (from 11.9 – 17.7%) of common variance.

It seems, therefore, that although changes in SDNN and HF mirror changes in RR interval in terms of mean effect size, mechanisms other than increased vagal modulation may be responsible for the resting bradycardia observed following a period of physical training. One such change may be reduced sympathetic activity, which is not strongly represented by either of the HRV measures analysed here. Other non-neural mechanisms which may influence resting HR

include alterations in the mechanical strain put on the pacemaker cells due to cardiac hypertrophy (Lewis *et al.* 1980) and metabolic alterations in pacemaker cells and neurotransmitter sensitivity (Ekblom *et al.* 1973; Katona and Jih 1975). It is thought that such adaptations are the result of prolonged exercise training and would be typically brought about by the relatively short exercise interventions used in the studies included here.

8.5.4. *Study limitations.*

The large degree of heterogeneity of the majority of measures analysed in the present study is a major limitation. Effect sizes for RR interval, SDNN and HF from cross-sectional studies, as well as RR interval and SDNN from longitudinal studies were all highly heterogeneous. These findings should, therefore, be interpreted with caution.

In both the cross-sectional and longitudinal analyses a relatively small number of studies were entered into the analysis providing small overall numbers of cases. This is due mainly to the small number of published studies in this area but also due to the frequent use of small sample sizes in this literature. The inability to perform a full meta-regression on the data weakens the conclusions which can be made concerning the factors responsible for the range of effect sizes shown in the literature. This is not so much shortcoming of the present study but of the literature cited which commonly fails to give the necessary information to facilitate such an analysis.

8.6. **Conclusions.**

The present data show clearly that resting bradycardia is evident from cross-sectional comparisons between individuals of differing physical activity levels. This difference is accompanied by increased vagal tone as indicated by HRV. This increased vagal modulation accounts for approximately half the variance in RR interval differences. A dose-response is evident, in as much as when athletes are compared to controls, the effect size is greater than when 'active' individuals

and controls are compared. In these comparisons, studies using 24-hour ambulatory HRV measurements showed a much greater overall effect size compared with those using short-term data collection. It should be noted, however, that only a small number of studies utilised the latter method.

In longitudinal analyses of change in RR interval, training induces a resting bradycardia, although the overall effect is less pronounced than in cross-sectional comparisons. This bradycardia is accompanied by increased global and vagally mediated measures of HRV. The latter, HF power, shows a homogenous positive response to training. Subgroup analysis of RR and SDNN reveal subject age and gender and previous level of physical activity can all influence the response to exercise. Additionally, it seems that in studies where short-term measures of HRV have been used, the effect size recorded is more than twice that of studies using 24-hour ambulatory HRV measures. However, the small number of studies using the latter methodology means caution should be employed prior to any generalisations being made regarding the superiority of one technique over the other.

8.6.1. Recommendations.

Increased availability of technology means that HRV studies are commonplace, however more randomised controlled studies would allow for a better quality of meta-analysis to be performed. The measurement of a 5-min ECG trace can be made very simply and analysed by commercially available instruments to give HRV measures. If this was done as an adjunct to the many hundreds of physical training studies carried out each year it would very simply and cheaply increase the available literature concerning HRV response to exercise. This would, in turn, help to clarify the role of the ANS in exercise-induced, resting bradycardia further.

Authors should report non-significant findings and report all HRV measures even if they are not tested statistically. Editors of journals should be encouraged to accept such findings for publication. Where HRV data are published this should be done in a manner which facilitates their inclusion in meta-analytical studies.

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CHAPTER 9. HEART RATE VARIABILITY IN PERIPHERAL ARTERY DISEASE.

Abstract.

The aim of this study was to determine whether HRV measured at rest and during exercise could be altered by an exercise training programme designed to increase walking performance in patients with peripheral artery disease.

Fifty-two volunteers were randomised into 12 weeks of either: supervised walking training (SU), home-based walking training (HB) or no exercise (CT). Recommended HRV measures were calculated from a 5-min resting ECG followed by maximal, graded exercise treadmill testing. All measures were repeated after 6 and 12 weeks. In the SU group, HRV measures were also recorded during 5-min of steady state exercise (75% baseline $\dot{V}O_{2\text{ peak}}$) at weeks 1, 6 and 12. The SU group showed significantly ($P < 0.001$) increased maximal walking time (MWT) but resting HRV measures remained unchanged in all groups. HRV measures repeated at equal absolute exercise intensities showed increases in RR interval, total and high frequency spectral power and the low-to-total spectral power ratio across the 12 weeks.

The lack of change in resting HRV was possibly due to either the low intensity or discontinuous nature of exercise undertaken. Alterations in exercise HRV suggest augmented cardioneuroregulatory response to exercise after training. Such measurements may illustrate training-induced changes in autonomic modulation when none are evident at rest.

This chapter, in truncated form has been published in The Journal of Exercise Science and Fitness and an abstract published in the Journal of Physiology, see appendix IV.

9.1. Introduction.

Peripheral arterial disease (PAD) is characterised by decreased peripheral blood flow and a low resting ankle-to-brachial blood pressure ratio or ankle-brachial index (ABI). The most common symptom reported by PAD patients is exercise-induced, ischaemic leg pain or intermittent claudication (IC) (Zatina *et al.* 1986). Symptomatic patients commonly demonstrate levels of peak exercise performance approximately half that of age-matched controls (Eldridge and Hossack 1987). This decreased capacity is associated with low levels of physical activity and low functional capacity (Hiatt *et al.* 1994).

The age-adjusted prevalence of PAD is approximately 12% (Criqui 2001). Only a quarter of patients diagnosed with PAD are symptomatic (Stewart *et al.* 2002). However, treatment remains of great importance, as patients have an increased risk of coronary and cerebrovascular event similar to that observed in patients with coronary artery disease (Dormandy *et al.* 1999).

Walking exercise results in large increases in claudication-free exercise time and maximal walking distance in PAD patients (Gardner and Poehlman 1995). However, a recent review (Stewart *et al.* 2002) suggested that the exact mechanisms by which this increase is facilitated remain unclear. There is evidence to suggest that increased efficiency, improved pain tolerance, improved gait, and increased limb blood flow may all contribute to increased exercise capacity. When compared with healthy controls, there is evidence of altered muscle carnitine metabolism during graded exercise (Hiatt *et al.* 1987; Hiatt *et al.* 1992). Altered ventilatory responses and slowed $\dot{V}O_2$ kinetics are also evident during steady state exercise in PAD patients, (Haouzi *et al.* 1997; Womack *et al.* 1997). There is some evidence of cardiopulmonary improvement due to exercise training (Hiatt *et al.* 1994) but such systemic adaptations are small when compared with changes in exercise capabilities (Hiatt *et al.* 1994; Regensteiner and Hiatt 1995; Falcone *et al.* 2003).

Information regarding systemic adaptation of the autonomic nervous system is less well documented (Hiatt *et al.* 1994). Evidence exists for skeletal muscle denervation in the affected limb of PAD patients (England *et al.* 1992). This has been linked to an attenuated temporal ventilatory response to exercise (Haouzi *et al.* 1997). However, at present, no empirical data are available concerning chronic, exercise-induced changes in autonomic function either at rest or during exercise.

9.1.1. *Can exercise training affect resting and exercise measures of HRV?*

9.1.1.1. The effects of exercise on HRV in healthy and clinical populations.

Chapter four provided a review of exercise and heart rate variability and chapter five provided a meta-analysis of these data. The conclusions drawn from these chapters were that exercise training in healthy populations increases global heart rate variability (HRV), vagal measures of HRV such as high frequency power (HF) and creates shifts in autonomic balance toward increased vagal modulation. Similar effects have been shown in a variety of clinical populations including post-myocardial infarction patients (chapter ten) and heart failure patients (chapter 11).

Data from longitudinal studies of autonomic nervous system (ANS) adaptation in healthy subjects are somewhat equivocal and several methodological issues are apparent (Aubert *et al.* 2003). Within such studies the lower threshold for exercise duration used is 30 minutes, the lower limit for exercise intensity is 60% of either $\dot{V}O_{2\max}$ or HR_{\max} . Considering this, two confounding factors may act to reduce or even negate the effects that aerobic exercise training may have on the ANS of PAD patients. Firstly, maximal absolute exercise intensities (W or METs) achieved by PAD patients are commonly reported to be approximately half that of healthy age-matched counterparts (Eldridge and Hossack 1987). This means that training intensities based on maximal achievable work rates will also be approximately half that of other subjects. Additionally, exercise duration is

commonly reduced due to IC in the periphery. This means PAD patients can often only exercise for short durations when compared with healthy subjects. Claudication-limited absolute exercise intensities and durations may, therefore, limit the overall systemic stress placed upon central systems of PAD patients during training. If such thresholds exist, below which central adaptations do not occur, then changes in ANS function due to exercise analogous to those observed in healthy individuals may not be realised in PAD patients.

In post-myocardial infarction patients, there is evidence that relatively low intensity exercise ($\sim 50\% \dot{V}O_{2\max}$ or HR_{\max}) may provide sufficient stimuli to promote cardiopulmonary adaptation. A recent meta-analysis suggested that present data provide no evidence of a lower exercise intensity threshold for increased exercise capacity and $\dot{V}O_{2\text{peak}}$ (Swain and Franklin 2002). There are, however, data to suggest a threshold effect of exercise training on alteration in autonomic function measured by HRV (Pardo *et al.* 2000). By dividing subjects into groups based on improvements in exercise capacity (< 1.5 METs vs. > 1.5 METs) Pardo *et al.* (2000) found significant improvements in HRV measures in the latter group only. Two points of criticism regarding this study should however be made. Firstly, the group analysis based on improvement in training was not one of the original aims of the study presented *a priori* and group allocation was carried out *pos hoc*. Secondly, there are data to suggest that improvement in exercise performance may be related to both initial levels and changes in certain HRV measures (Boutcher and Stein 1995; Hautala *et al.* 2003). As the authors did not control for initial levels of HRV between groups, generalisations based on these findings should be made with caution.

On the basis of existing data regarding the adaptation of HRV measures to exercise training, the first two aims of this chapter were to ascertain the following:

- i. Whether resting measures of short-term HRV were attenuated in PAD patients as they are in other ischaemic disease states.

- ii. Whether HRV could be improved in PAD patients receiving 12 weeks of supervised or home based exercise training compared to a control group receiving no treatment.

9.1.1.2. Chronic changes in HRV during exercise.

There are some cross-sectional data in healthy populations to suggest that HRV measurements made during exercise are affected by levels of habitual physical activity (Gregoire *et al.* 1996; Levy *et al.* 1998; Myslivecek *et al.* 2002). There are limited longitudinal data supporting the notion of a role for exercise training in modifying HRV measures made during exercise (Myslivecek *et al.* 2002). The findings of such studies are, however, heterogeneous (Perini *et al.* 2002). The validity of the research cited above may have been negatively affected by the use of HRV measurements not previously validated for use during exercise. The third aim of this chapter was, therefore:

- iii. To determine whether steady-state exercise HRV measurements could be altered following a supervised treadmill walking programme in PAD patients.

9.1.2. Changes in exercise performance in PAD patients.

Exercise training studies in PAD patients unanimously report increased walking performance variables. The findings of these studies have been reviewed (Regensteiner and Hiatt 1995; Hiatt 2001; Stewart *et al.* 2002; Kugler and Rudofsky 2003; Nehler *et al.* 2003) and meta-analysed (Gardner and Poehlman 1995) previously. Increases in time for pain-free walking range from 44% – 300%. And values for maximal walking distance range from 25% - 442% (Nehler *et al.* 2003). A systematic review of these studies is beyond the scope of this chapter, but two hospital-based, supervised, randomised controlled trials are of particular merit. In one study (Hiatt *et al.* 1990) patients were randomly assigned to walking exercise or non-exercise control. After three months of progressive treadmill walking (one hour, three times a week) maximal walk time increased 123% and pain-free walk time increased 165% in the exercise group.

Controls showed only a 20% increase in walk time over the same period. The same research group (Hiatt *et al.* 1994) later demonstrated similar improvements (128% increase in maximal walk time) in exercise capacity and demonstrated a greater efficacy of treadmill walking vs. strength training for increasing walking performance. Improved functional capacity and increased levels of habitual physical activity were also recorded in the patients receiving the treadmill walking exercise protocol.

9.1.3. *Central vs. peripheral changes: which are responsible for increased walking performance?*

The magnitude of increases in walking performance with exercise training, are commonly in excess of those for changes in central factors such as $\dot{V}O_{2\text{ peak}}$. For example, Hiatt *et al.* (1990) found that the respective increases of 123% and 165% for pain free and maximal walk time were associated with only small (30%) increases in $\dot{V}O_{2\text{ peak}}$. Changes in $\dot{V}O_{2\text{ peak}}$ are unanimously reported as being much smaller in magnitude than changes in walking performance (Hiatt *et al.* 1990; Hiatt *et al.* 1994) suggesting other factors must be responsible in part for the large increase commonly observed. In an attempt to identify these factors, changes in a number of measures over the course of exercise training protocols have been studied. Improved walking economy, efficiency and altered gait have all been reported (Womack *et al.* 1997). Such adaptations facilitate improvements in walking performance independent of changes in $\dot{V}O_{2\text{ peak}}$ (Hiatt *et al.* 1990; Hiatt *et al.* 1994).

Studies concerning change in peripheral arterial blood flow during exercise and more commonly, resting measures of ABI, have provided mixed results concerning the role of leg blood flow in improved walking performance. Despite IC being due to anaerobiosis in the peripheral musculature, the majority of ATP resynthesis during exercise in claudicants remains aerobic (Green and Mehlsen 1999). On this basis, leg blood flow should be a determinant of walking performance. Furthermore it would be expected that changes in walking

performance would be related to changes in leg blood flow. This, however appears not to be the case.

Using resting ABI as an indicator of potential muscle blood flow, there is a large body of evidence to suggest that muscle blood flow is not related to onset of claudication or maximal walking performance (Gardner *et al.* 1992; Hiatt *et al.* 1992; Brass and Hiatt 2000). Additionally, improvements in ABI due to bypass surgery and/or exercise do not correlate with increases in walking performance (Regensteiner *et al.* 1993). In healthy individuals ABI values at rest are > 1 . A value of < 0.94 indicates reduced blood flow to the lower limb at rest and is used as a diagnostic marker of PAD. Percutaneous angioplasty has been shown to increase resting ABI (from 0.6 to 0.9) in the absence of any improvement in walking performance (Perkins *et al.* 1996). Further evidence for the dissociation of limb blood flow and walking performance comes from studies showing large improvements in walking performance with no concomitant changes in ABI (Hiatt *et al.* 1990; Hiatt *et al.* 1994).

It seems therefore, that change in resting ABI is an unlikely candidate to explain improved walking performance. The majority of studies show little-or-no change in ABI following exercise (Green 2002) and where significant changes in ABI are reported, they do not correlate with changes in walking performance (Womack *et al.* 1997). Data derived via the superior technique of measuring dynamic leg blood flow during exercise are very limited (Green 2002) and provide results which are at best, equivocal.

9.1.4. *Other possible mechanisms for improved walking performance.*

There is little evidence of impaired oxygen extraction across the affected limb in PAD patients. However, alterations in muscle metabolism have been demonstrated (Hiatt *et al.* 1987; Hiatt *et al.* 1992; Okita *et al.* 1998). In common with ABI measurements and $\dot{V}O_{2 \text{ peak}}$ data, these measures correlate only weakly with walking performance. No longitudinal data are available to determine

whether changes in muscle metabolism correlate with changes in maximal walking distance.

Little attention has been paid to the possible contribution of impaired cardiac function to reduced exercise capacity. The absence of angina and electrocardiographic abnormalities during exercise has been interpreted as evidence that they are not limiting factors in exercise performance. However, the screening, and consequent exclusion from studies of patients displaying these characteristics means data will undoubtedly be biased toward such an interpretation. The existing data suggest cardiac function does not limit exercise performance. Peak heart rates are commonly only 100 – 125 BPM (Hiatt *et al.* 1990; Gardner *et al.* 1992; Hiatt *et al.* 1994). Although these may increase following training they remain severely attenuated when compared to healthy age-matched controls (Martin *et al.* 1990). Such submaximal values suggest that peripheral rather than central factors are responsible for reduced exercise performance in PAD patients. Further evidence to support this argument is provided by the failure of PAD patients to attain a plateau in oxygen consumption during incremental exercise testing (Eldridge and Hossack 1987). Some patients can, however, attain a plateau and have previously been found to attain higher heart rates (and %HR_{max}) than those who fail to demonstrate such a pattern (Eldridge and Hossack 1987). These authors concluded that a lack of sympathetic drive to the heart may be responsible for the non-attainment of a classical plateau in oxygen consumption. No measure of sympathetic nervous activity was made in this study.

Evidence for denervation of the ischaemic limb in PAD patients also exists (England *et al.* 1992). It may be that reduced feedback from peripheral chemoreceptors and mechanoreceptors is responsible for the low reactance of the cardiovascular system to exercise (low maximal HR). It is unlikely that this central factor is the primary determinant of exercise performance in PAD patients but its contribution has not yet been quantified. Additionally, in the absence of one single factor being identified as being responsible for reduced walking performance, quantification of the contribution from as many central and peripheral factors as possible is warranted. Similarly, quantification of the

adaptation of any determinants to exercise training may help to elucidate the factors which limit exercise performance in PAD patients. The final aim of this chapter was, therefore:

- iv. To identify possible predictive measures of improved walking performance from resting and exercise baseline measurement.

9.2. Methods.

9.2.1. Study population.

Subjects were 52 consecutive patients (38 males, mean age 64 ± 9) years from the vascular outpatient clinic who gave written, informed consent. The study was approved by the Hillingdon Hospital local research ethics committee. Peripheral artery disease was confirmed in all subjects by an ankle-to-brachial index < 0.94 at rest using a Doppler system (Mini Dopplex, Model 2000, Huntleigh Diagnostics, Cardiff, UK). The presence of symptomatic IC was evaluated using the leg pain score (ACSM 2000).

9.2.2. Protocol.

9.2.2.1. Resting heart rate variability analysis.

All subjects underwent a five minute resting ECG recording using a standard 12-lead ECG (CardioPerfect ST 2001, Cardio Control, Delft, The Netherlands). Heart rate variability was calculated according to recommended standards (Task Force 1996) using the method described previously (Chapters: 2 and 3) except that heart rate was recorded with subjects in the semi-recumbent position in a comfortable chair as opposed to in the supine position.

9.2.2.2. Exercise heart rate variability measurements.

In subjects allocated to the supervised exercise condition heart rate variability was measured during exercise using both recommended measures (Task Force

1996) and those developed specifically for use during exercise (Warren *et al.* 1997). Five minute ECG recordings were made during steady state exercise at a workload equivalent to 75% of each subject's maximum. Initial measurements were only undertaken when it was deemed possible for a subject to attain and maintain steady state conditions for this duration of exercise. Exercise HRV measures were repeated at the same absolute exercise intensity after 6 and 12 weeks of exercise training. If subject exercise capacity had improved sufficiently at these time points, exercise HRV was also recorded at the new relative exercise intensity.

9.2.2.3. Treadmill testing protocol.

Subjects were first given a treadmill familiarisation session on the day of testing followed by seated rest. After $\dot{V}O_2$ and heart rate returned to baseline values, subjects completed a graded treadmill protocol recommended for use in PAD patients (Gardner *et al.* 1991). Subjects walked on a treadmill (Marquette 2000, Marquette Electronics, Milwaukee, WI) at an initial workload of 2 mph for 2 minutes. Consequent stages of the test increased 2% in grade every 2 minutes. The reliability of treadmill test scores, clinical measurements during and after this test have been demonstrated previously (Gardner *et al.* 1991; Labs *et al.* 1999). During exercise, heart rate was measured and recorded via 12-lead ECG, (CardioPerfect ST 2001, Cardio Control, Delft, The Netherlands) $\dot{V}O_2$ and $\dot{V}CO_2$ measurements were made breath-by-breath, using a Medical Graphics Cardio₂ analysis system (Medical Graphics Corporation, St. Paul, Minnesota) and blood pressure was measured by manual sphygmomanometry. Onset, change and severity of IC were assessed in the final minute of each stage using the leg pain scale (ACSM 2000). This scale rates claudication pain from 1 (mild discomfort) to 4 (excruciating and unbearable pain). It is recognised that pain stimuli may modulate selected HRV measures in (Lind *et al.* 1999). On this basis, IC may be viewed as a potential confounding variable. However, the magnitude of effect exercise exerts on HRV is much greater than that reported for pain. Also, as pain cannot be avoided in this population, it was adjudged that best practice was to monitor pain so that it may be controlled for, if necessary in

later analyses. Additionally, ratings of perceived exertion were measured in the last minute of each stage. Treadmill testing and all associated resting and exercise measurements were repeated in all groups after six and twelve weeks.

9.2.2.4. Test termination criteria.

The following exercise test termination criteria were employed. Volitional exhaustion, sustained ST segment depression > 2 mm, acute chest pain, acute leg pain other than that of ischaemic origin or achievement of $\dot{V}O_{2\text{ max}}$. Maximal oxygen consumption was defined in two ways. In the first condition, three criteria were employed based on those described by the ACSM (2001). These were:

- i. a plateau in $\dot{V}O_2$ (increase of $< 2 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) with an increase in workload,
- ii. a respiratory exchange ratio > 1.15 ,
- iii. an RPE > 17 .

Second, $\dot{V}O_{2\text{ max}}$ was characterised by a plateau in oxygen consumption only as described in this population previously (Eldridge and Hossack 1987). *Post hoc* analysis of respiratory data was also carried out using automated software (Breeze Suite. Medical Graphics Corporation, St. Paul Minnesota). Standard nine-panel-plots were created by which achievement of anaerobic threshold (AnT) and $\dot{V}O_{2\text{ max}}$ could be determined.

9.2.2.5. Randomisation and group exercise protocols.

The remaining subjects were randomly allocated to one of three conditions: Supervised exercise training (SU), home based exercise training (HB) or non exercising controls (CT.) The supervised group attended the hospital twice a week. During each session they were required to complete a total of 30 min treadmill walking at 75% of maximum walking capacity. They were also instructed to undertake one additional weekly unsupervised walking session of 30 min duration at an intensity eliciting an RPE of 11-13 at home between visits. The intensity of exercise was titrated by the researchers to account for improvements in exercise tolerance and performance during the trial using the

ratings of perceived exertion (RPE) scale (Noble *et al.* 1983) and measurements taken during retesting. The home-based exercise group were asked to complete an exercise diary and instructed to walk for total of 30 min three times a week for the duration of the study. They were given a copy of the RPE scale and instructed to titrate their exercise intensity to a level equivalent to between levels 11 and 13. Weekly follow-up telephone calls were made by the researchers to discuss the protocol with subjects, answer any questions they might have and to give encouragement in trying to maintain the protocol. The control group were asked to maintain their current level of physical activity throughout the 12 weeks of the study.

9.2.3. *Statistical analysis.*

All measures were checked for normality of distribution and all underlying assumptions for each statistical test were assessed thoroughly before test application. Where data transformations or non-parametric equivalent tests were used these are outlined in individual results sections.

9.2.3.1. Changes in resting measures of heart rate variability.

Changes in heart rate variability measures between groups over the 12 weeks were analysed using a mixed (3 x 3) analysis of variance with repeated measures (mixedANOVA). *Post hoc* analysis between groups was carried out using the test for least significant differences (LSD). *Post hoc* analysis within groups was carried out using repeated measures *t*-test with a Bonferroni correction factor for multiple comparisons. Due to the large number of potential comparisons, only selected variables were analysed statistically. First mean RR interval was compared to determine whether evidence of exercise training-induced resting bradycardia was present. Global HRV was assessed in the time domain using the standard deviation of normal to normal intervals (SDNN). High frequency spectral power (HF) was used as a marker of vagal modulation. Sympathetic modulation was measured using low frequency power expressed in normalised units (LFnu) and LF: HF ratio was used as a marker of sympathovagal balance.

9.2.3.2. Changes in measures of heart rate variability made during exercise.

Only data from the SU group were entered into this analysis. Heart rate variability measures validated for use during exercise (Warren *et al.* 1997) were calculated. Each selected variable was then analysed using repeated-measures analysis of variance (rmANOVA) with *post hoc* analysis (rm *t*-test).

9.2.3.3. Baseline measures as predictors of maximal walking distance at baseline and change in maximal walking distance at 12 weeks.

To identify those factors which best predicted maximal walk time (MWT) and change in maximal walking time (Δ MWT), multiple stepwise regression analysis was used. Inclusion criteria for predictor variables were a significant ($P < 0.05$) effect on F in analysis of variance, removal occurred at ($P > 0.10$).

9.3. Results.

Forty-nine patients were deemed suitable for enrolment into the study. One patient was excluded due to unstable angina symptoms. Two were rejected due to symptoms associated with critical ischaemia during treadmill familiarisation. After preliminary exercise testing a further two patients were excluded due to being unable to walk at 2 mph on level ground and one for displaying consistent ST elevation.

One subject was admitted for elective peripheral bypass surgery after 6 weeks of exercise and a further patient suffered an acute ischaemic cerebral event at week 11. Full data sets at baseline and six weeks were therefore, available in 40 subjects. Complete study data were available on 39 subjects.

9.3.1. Baseline data.

Baseline subject characteristics are given in table 9-1, baseline exercise test data and changes in test data at weeks six and twelve are shown in table 9-2.

Table 9-1. Descriptive characteristics by group.

Group	Age (yrs)	Height (cm)	Mass (kg)	BMI (kg/m ²)	ABI
SU	66 ± 8	1.70 ± 0.12	76.9 ± 18.3	26.3 ± 4.5	0.59 ± 0.01
HB	62 ± 14	1.69 ± 0.11	79.3 ± 13.0	27.1 ± 4.2	0.57 ± 0.01
CT	67 ± 6	1.71 ± 0.08	81.3 ± 22.5	27.7 ± 6.7	0.57 ± 0.12

SU - Supervised exercise, HB - Home based, CT- Control, BMI - Body mass index, ABI - Ankle-to-brachial index.

Baseline exercise test data (table 9-2) showed low values for maximal walking times and levels of $\dot{V}O_{2\text{ peak}}$. At baseline, all exercise test data were similar between groups. No significant changes in physiological measures or pain ratings occurred in any group. There was, however a significant ($P < 0.0001$) improvement in MWT in the SU group from baseline to week six. This value continued to increase ($p = ns$) from week six to week twelve.

Baseline HRV data in table 9-3 show severely attenuated levels of global HRV characteristics (TP and SDNN) as well as low values for HF. High mean values for LF:TP and LFnu were also evident. One-sided *t*-tests showed that SDNN, TP and HF were all significantly lower than those reported for healthy subjects and similar to those reported previously for post-MI patients.

Table 9-2 Exercise test data at baseline, six and twelve weeks.

Group	MWT (min)	$\dot{V}O_2$ peak (ml • kg ⁻¹ • min ⁻¹)	RER	RPE	Pain rating
SU 1	6.50 ± 4.0	14.2 ± 3.8	0.96 ± 0.10	16.0 ± 2.0	3.0 ± 0.5
SU 6	12.0 ± 5.6*	14.3 ± 3.7	1.00 ± 0.10	15.0 ± 2.5	3.0 ± 1.0
SU 12	12.1 ± 6.3*	13.7 ± 4.2	0.99 ± 0.12	15.1 ± 2.4	2.8 ± 0.9
HB 1	6.1 ± 3.3	14.3 ± 4.1	1.03 ± 0.08	16.0 ± 2.0	3.0 ± 1.0
HB 6	7.1 ± 4.7	14.0 ± 4.4	1.01 ± 0.10	16.0 ± 2.5	3.0 ± 1.0
HB 12	7.2 ± 5.2	13.7 ± 4.1	1.03 ± 0.99	16.1 ± 2.5	2.77 ± 0.9
CT 1	6.4 ± 4.4	14.8 ± 4.7	1.00 ± 0.13	15.0 ± 2.0	3.0 ± 0.5
CT 6	6.3 ± 5.2	14.5 ± 3.9	0.99 ± 0.07	14.7 ± 2.5	3.0 ± 1.0
CT 12	7.1 ± 7.1	14.3 ± 5.1	0.99 ± 0.09	14.3 ± 5.1	2.6 ± 0.7

1 - week one, baseline, 6 - week six, 12 - week twelve, SU - Supervised exercise, HB - Home based, CT- Control, MWT - Maximal, walking time, RPE - Rating of perceived exertion, RER - Respiratory exchange ratio

* Significantly different from baseline. ($P < 0.001$)

9.3.2. *Changes in resting HRV due to exercise training.*

Mixed ANOVA showed no significant overall effect of exercise training and no group interaction for any of the selected HRV measures (see table 9-3). *Post hoc* analysis (LSD) showed no differences between SU, HB and CT groups at baseline for any HRV measure. Further within-group, *post hoc* analysis over time revealed no significant effects of exercise in any group.

9.3.2.1. Changes during exercise at equal absolute exercise intensity.

This analysis was carried out on the SU group only ($n = 14$). The three HRV measures used differed from those analysed in previous sections. Due to the differences in chosen spectral parameters, it was first necessary to assess the changes these effects had on resting HRV measures. One-way ANOVA revealed no significant effects of exercise training on the resting HRV measures: TP, (0.003 - 0.1 Hz) HF, (0.1 - 1.0 Hz), or LF:TP ratio.

Data from HRV measured during exercise are displayed in figures 9-1 – 9-4. When measured at the same absolute exercise intensity, rm-ANOVA revealed a significant change in RR interval over time ($F = 4.30$, $P = 0.028$). *Post hoc* analysis revealed an increase in RR interval from baseline to week six ($t = 2.19$, $P = 0.079$) and a significant difference between baseline and week 12 ($t = 3.58$, $P = 0.004$). There was a significant effect of exercise on TP ($F = 4.44$, $P = 0.024$). *Post-hoc* analysis (rm *t*-test) revealed significant differences in TP from baseline at weeks six ($t = 2.62$, $P = 0.021$) and 12 ($t = 3.32$, $P = 0.006$).

Table 9-3. Heart rate variability measures at baseline, six and twelve weeks.

Group	HF (ln)	LFnu	LF:HF (ln)	RR interval
SU 1	4.1 ± 1.3	64.4 ± 22.0	0.73 ± 1.22	756.6 ± 154.3
SU 6	4.4 ± 1.6	70.3 ± 21.2	0.99 ± 1.00	766.3 ± 114.5
SU 12	3.9 ± 1.2	68.4 ± 14.3	0.91 ± 0.94	799.8 ± 144.6
HB1	3.6 ± 1.3	67.7 ± 21.2	0.73 ± 1.02	773.0 ± 213.0
HB 6	3.3 ± 1.4	75.9 ± 15.3	1.28 ± 0.82	748.1 ± 201.8
HB 12	3.9 ± 1.3	66.3 ± 14.3	0.76 ± 0.71	808.4 ± 177.0
CT 1	4.0 ± 1.4	79.6 ± 20.0	1.50 ± 0.68	840.5 ± 118.2
CT 6	4.4 ± 1.4	72.5 ± 18.8	1.14 ± 0.77	824.4 ± 136.7
CT 12	4.2 ± 1.3	68.7 ± 24.8	1.51 ± 1.19	836.6 ± 146.7

1 - week one, baseline, 6 - week six, 12 - week 12, SU - Supervised exercise, HB - Home based, CT- Control, HF(ln) - High frequency spectral power in log transformed units, LFnu - Low frequency power in normalised units, LF:HF - the ratio of low to high frequency power

High frequency power during exercise was increased by exercise training ($F = 3.22$, $P = 0.053$). *Post hoc* analysis (rm t-test) showed significant differences in HF from baseline at weeks six ($t = 3.22$, $P = 0.007$) and 12 ($t = 2.50$, $P = 0.030$). The ratio of LF:TP showed no significant effect over time ($F = 1.15$, $P = 0.336$). *Post hoc* analysis revealed a significant increase in LF:TP from baseline to 12 weeks ($t = 2.36$, $P = 0.036$).

9.3.2.2. Changes during exercise at equal relative exercise intensity.

When measured at equal relative exercise intensity (75% $\dot{V}O_{2\text{ peak}}$) there were no significant changes from baseline in RR interval, TP, HF or LF:TP in the SU group at week six or week 12.

9.3.3. *Predictors of baseline maximal walking time.*

Ranges for maximal walking times are shown in table 9-2. When anthropometric, clinical, exercise test and HRV measures were used to predict MWT in all subjects tested at baseline $\dot{V}O_{2\text{ peak}}$ was found to be the only significant predictor (See equation 9-1).

$$\Delta\text{MWT} = (39.3 \times \dot{V}O_{2\text{ peak}}) - 199.5 \quad \text{Equation 9-1}$$

$R^2 = 0.51$ and $\text{SEE} = 167.8$

9.3.4. *Predictors of change in maximal walking time in SU group.*

With the SU group's ΔMWT scores as the dependent variable, stepwise regression revealed that baseline $\dot{V}O_{2\text{ peak}}$ and HF(ln) were both significant independent predictors (See equation 9-2).

$$\Delta\text{MWT} = (23.2 \dot{V}O_{2\text{ peak}}) + (63.3 \text{ HF}(\ln)) - 511.4 \quad \text{Equation 9-2}$$

$R^2 = 0.74$, $\text{SEE} = 90.9$

9.3.5. *Predictors of change in maximal walking time in all patients.*

With the all patient's Δ MWT scores as the dependent variable, stepwise regression revealed that baseline $\dot{V}O_{2\text{ peak}}$ and HF(ln) were both significant independent predictors (See equation 9-3).

$$\Delta\text{MWT} = (24.2 \dot{V}O_{2\text{ peak}}) + (52.1 \text{ HF}(\ln)) - 511.4 \quad \text{Equation 9-3}$$

$$R^2 = 0.27, \text{ SEE} = 219.3$$

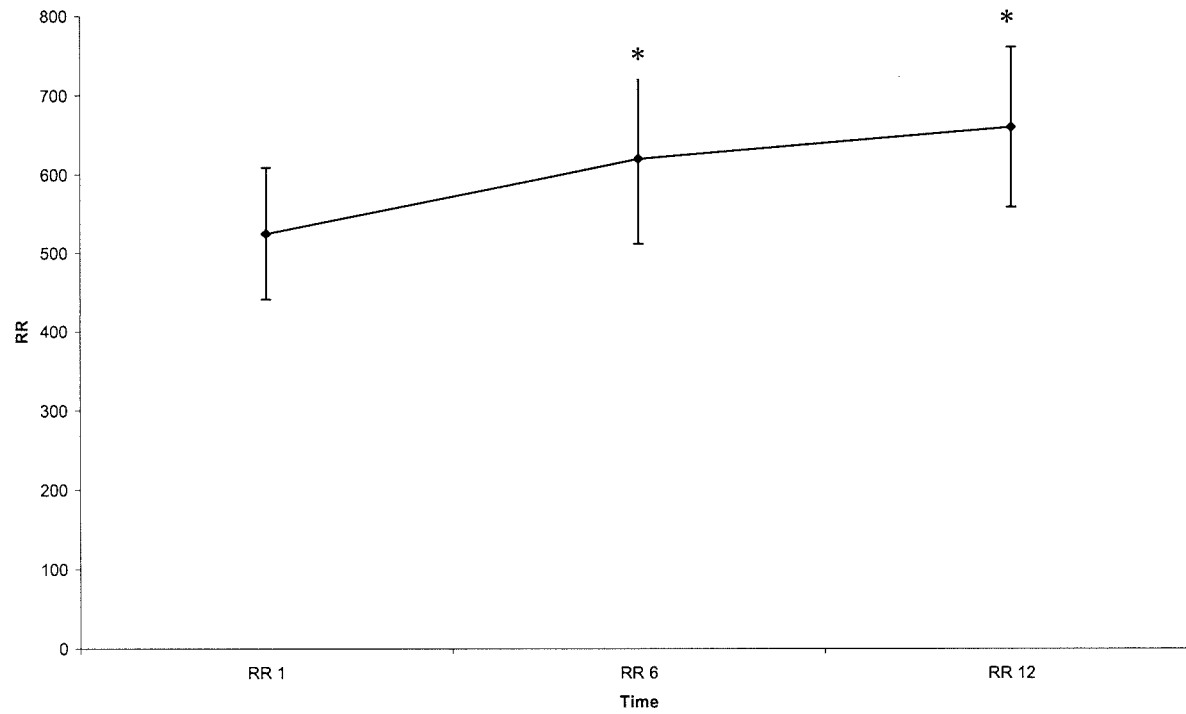


Figure 9-1. Mean RR interval during exercise at the same absolute exercise intensity at weeks one, six and twelve.

RR – mean RR interval (ms), RR 1 – baseline, RR 6 – week six, RR 12 - week twelve. * - significantly ($P < 0.05$) different from baseline.

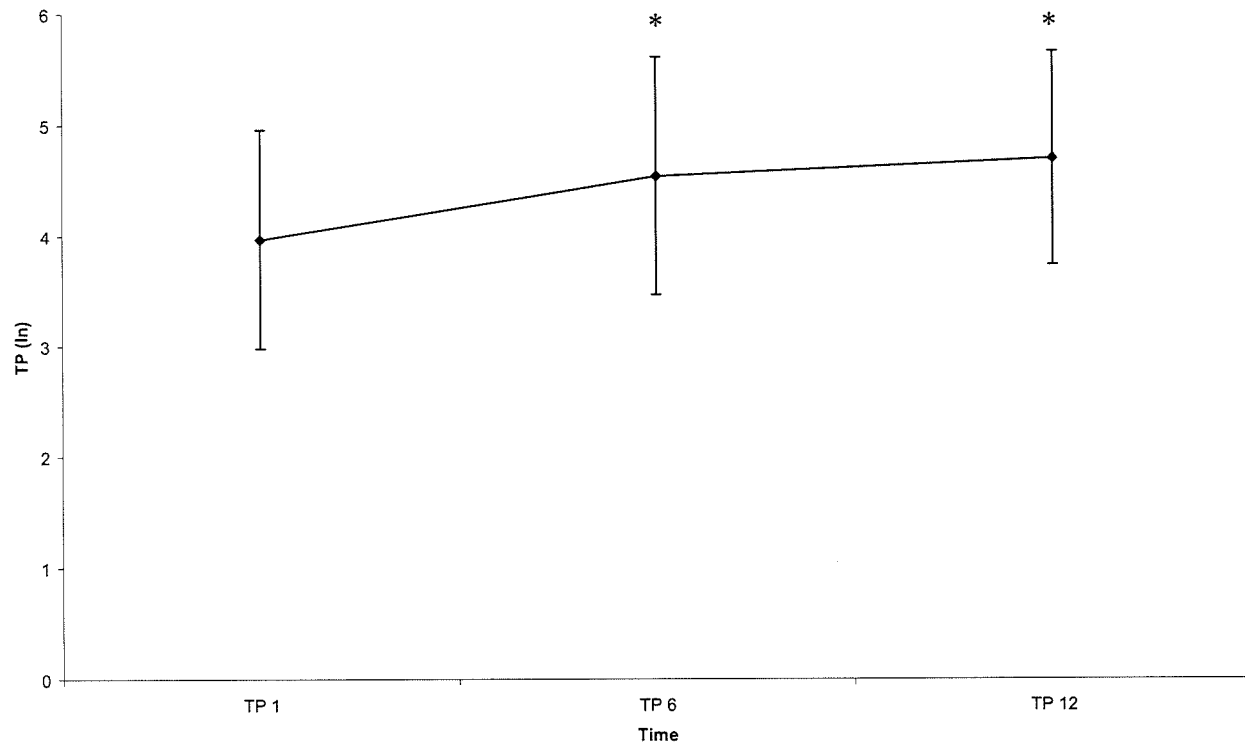


Figure 9-2. Total spectral power during exercise at the same absolute exercise intensity at weeks one, six and twelve.

TP - mean total spectral power (ms²), TP 1 – baseline, TP 6 – week six, TP 12 - week twelve. * - significantly (P < 0.05) different from baseline.

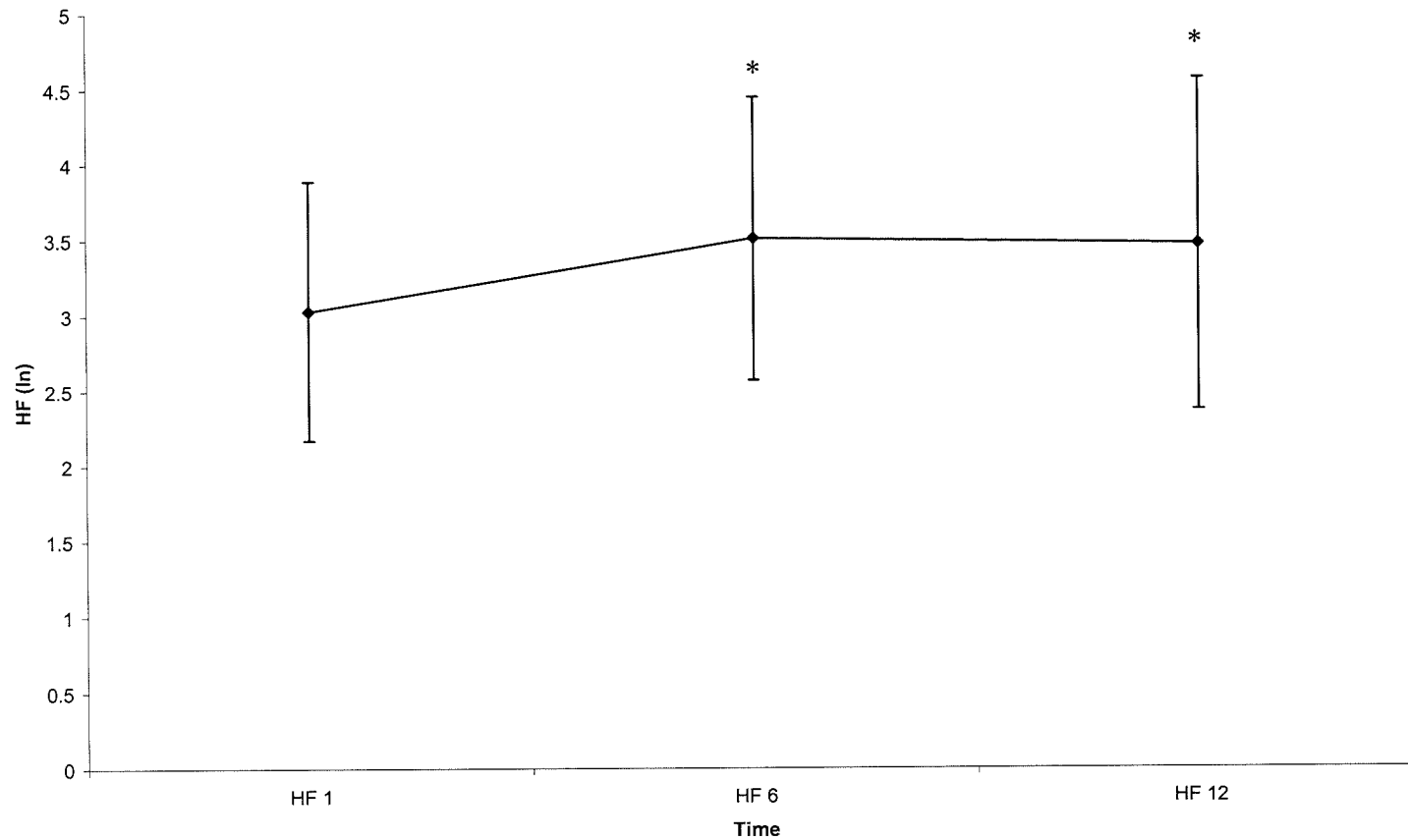


Figure 9-3 High frequency spectral power (log units) during exercise at the same absolute exercise intensity at weeks one, six and twelve.

HF (ln) - mean high frequency spectral power (log units), HF 1 – baseline, HF 6 – week six, HF 12 - week twelve. * - significantly ($P < 0.05$) different from baseline

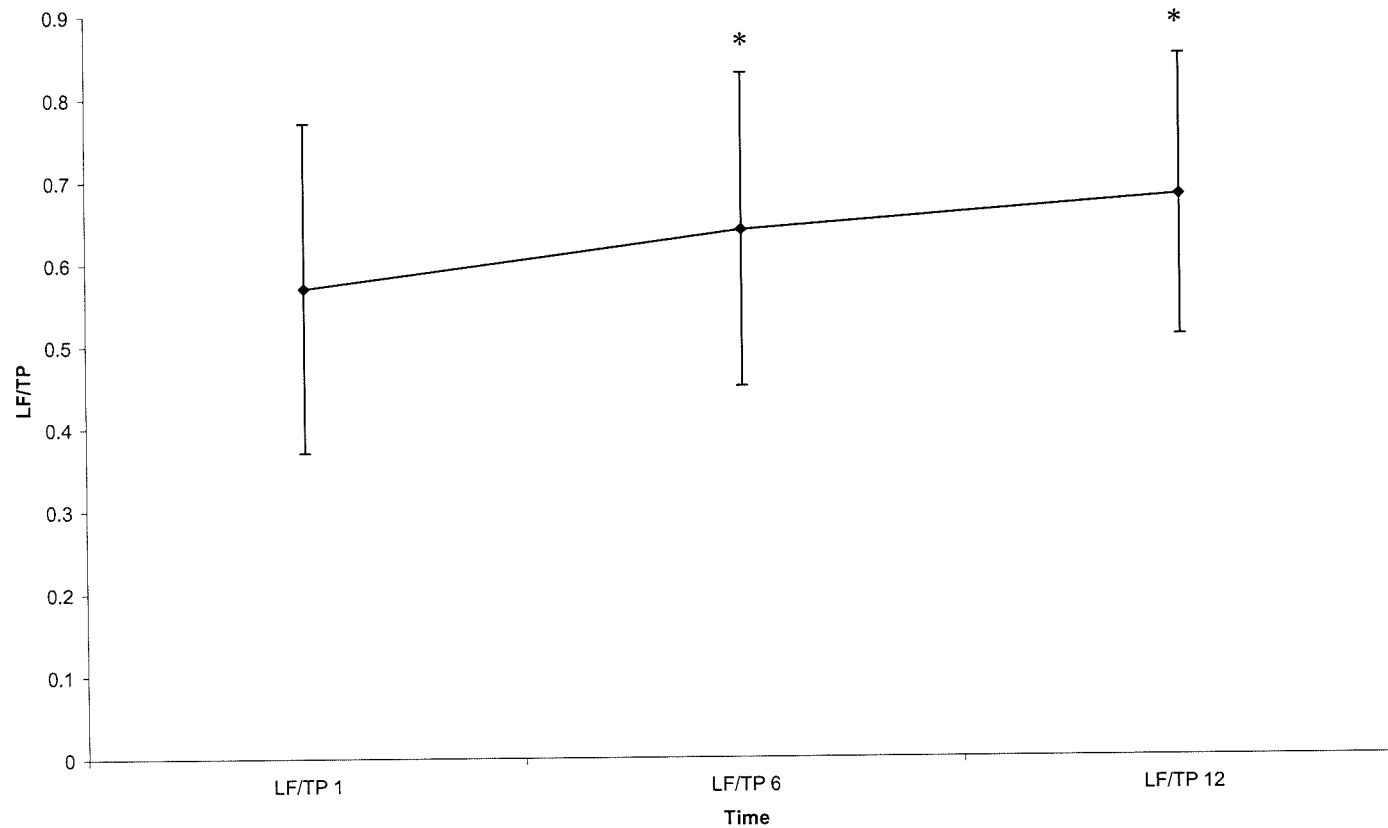


Figure 9-4 Ratio of low frequency to total spectral power during exercise at the same absolute exercise intensity at weeks one, six and twelve.

LF/TP- mean ratio of low frequency to total spectral power (arbitrary units), LF/TP 1 – baseline, LF/TP 6 – week six, LF/TP 12 - week twelve. * - significantly ($P < 0.05$) different from baseline.

9.4. Discussion.

9.4.1. Exercise test and HRV data.

Values for MWT and $\dot{V}O_{2\text{ peak}}$ in the present PAD patient cohort were similar to those reported previously for incremental treadmill testing in clinically similar patient groups (Regensteiner *et al.* 1993; Hiatt *et al.* 1994; Gardner 2002). These data support statements made in several review articles which conclude that exercise performance and functional capacity are severely attenuated in PAD patients (Gardner and Poehlman 1995; Regensteiner and Hiatt 1995).

To date, no data are available concerning the cardioneuroregulatory status of PAD patients. In ischaemic conditions such as angina, 24-hour monitoring has shown that when compared to controls, angina patients have significantly reduced mean RR interval, SDNN, the root mean square of successive RR intervals (RMSSD), the number of adjacent beats differing by more than 50 ms in length (pNN50), TP, LF, HF (Wennerblom *et al.* 2000). Some measures are also reduced when calculated from short-term ECG traces. When calculated from 24-hour monitoring, HF, LF and VLF all predict cardiac death in stable angina patients (Forslund *et al.* 2002) and the LF:HF ratio is also known to be a powerful predictor of cardiac event in unstable angina (Lanza *et al.* 1997). Wennerblom *et al.* (2000) found that Percutaneous transluminal coronary angioplasty (PTCA) aided the recovery of attenuated vagal measures of HRV (HF) calculated from short-term ECG measurement under controlled respiration. This medical procedure, however, failed to normalise all HRV measures, indicating that ischaemia itself, was one, but not the only cause of the disturbed HRV in stable angina.

Short-term values for SDNN were similar to those reported previously (Faber *et al.* 1996) in a subgroup of post-MI patients, identified as being at high risk of three

year, all cause mortality. Additionally, values for HF were similar in PAD patients and high risk post-MI patients. Finally, when compared with values derived from similar short-term ECG measurements in subjects of similar age (Barnett *et al.* 1999), PAD patients had greatly reduced values for all HRV measures, but particularly global (SDNN) and vagal (HF) measures (Faber *et al.* 1996; Fei *et al.* 1996). These findings indicate that the PAD patients studied here may be at increased risk of future cardiac event and overall mortality compared with the normal population.

9.4.2. *Changes due to exercise training.*

The CT group in the present study were given no specific exercise therapy and only instruction of the effects of exercise on PAD other than that provided by their general practitioner and their hospital consultant. No differences were found in any of the measures recorded in the study and therefore the discussion will be limited to data from the SU and HB groups.

9.4.2.1. Changes in walking performance and $\dot{V}O_{2\text{ peak}}$ in SU and HB groups.

The efficacy of supervised walking exercise in increasing walking capacity in PAD patients has been demonstrated in numerous studies and reviewed thoroughly (Regensteiner and Hiatt 1995). The large improvements over the 12 weeks of exercise training in the SU group agree with findings from previous randomised controlled trials (Hiatt *et al.* 1990; Hiatt *et al.* 1994). Data from community-based exercise programmes demonstrate them to be less efficacious. Overall, however, they still bring about increases in walking performance. The present data agree with these findings, they also agree in part, with previous data suggesting that changes in MWT are not always associated with any significant change in $\dot{V}O_{2\text{ peak}}$ (Kugler and Rudofsky 2003). This has not always been shown to be the case however, as

significant increases in central cardiopulmonary capacity have been demonstrated (Regensteiner and Hiatt 1995).

9.4.2.2. Changes in resting HRV in SU and HB groups.

No previous data were available concerning the effectiveness of exercise training as an intervention to alter the HRV profiles of PAD patients. Based on the low values recorded at baseline and the risk associated with such values in other populations it seems logical to argue that increases in global and especially vagal measures of HRV would be advantageous to these patients. It is known that exercise training can positively affect the HRV profiles of post-MI patients (La Rovere *et al.* 1992; Stahle *et al.* 1999), and even heart failure patients (Coats *et al.* 1992). Typical effects appear to be increases in global measures of HRV such as TP in the frequency domain and SDNN in the time domain. Additionally, a shift toward increased parasympathetic modulation of HR has been demonstrated (Mazzuero *et al.* 1992). These changes are similar to those observed in healthy individuals who undertake exercise training (See Chapters four and five for full reviews).

In the present study, the HB group showed no significant effects of exercise training on HRV. This was expected as no increases in either exercise performance (MWT) or cardiopulmonary function ($\dot{V}O_{2\text{ peak}}$) were detected. In the majority of studies where alterations in HRV are demonstrated this is commonly observed in conjunction with significant alterations in other physiological measures. Data from post-MI patients suggest evidence of a threshold effect in the ability of exercise to alter HRV significantly (Pardo *et al.* 2000). These authors found no effect of exercise rehabilitation in post-MI patients when analysed by group (home-based group and supervised exercise group). In their study, Pardo *et al.* (2000) originally utilised the home-based exercise group as a control group. When it was discovered that several 'controls' had made significant > 1.5 MET increases in maximal exercise capacity their data were pooled with those of subjects in the experimental

arm of the study. These ‘responders’ as they are termed were then compared with the ‘non-responders’; defined as those subjects improving by < 1.5 METS maximal exercise capacity. There are several methodological issues associated with such *post hoc* data pooling techniques especially in analysing pooled data from different subject groups who have received different interventions. Therefore, although these researchers found differences in HRV measures between responders and non-responders it is difficult to infer causality from such findings.

Interestingly, in the present study, several HB group subjects and one CT group subject showed large increases in exercise capacity and indeed $\dot{V}O_{2\text{ peak}}$. On this basis it was decided that two analyses ‘without intention to treat’ be performed. The first was to determine if any differences were evident at baseline between responders and non-responders. By dividing subjects according to improvement in MWT (< 280 s = non responder or > 280 s = responder) on all patient data to determine factors which may predict improvement in MWT independent *t*-test showed those who responded to exercise training (in HB and SU groups) had higher levels of HF(ln) recorded at baseline (data not shown). These findings are in agreement with data from studies in healthy individuals (Boutcher and Stein 1995; Hedelin *et al.* 2001; Hautala *et al.* 2003). Boutcher and Stein (1995) studied the absolute and relative $\dot{V}O_{2\text{ peak}}$ of 15 controls and 19 subjects who trained for 24 sessions. These two values increased 12% and 11% respectively, no increase was observed in the control group. As in the present study, HRV remained unchanged. However, when the trained subjects were further categorised into high ($n = 5$) and low ($n = 5$) HRV at baseline groups improvements in both absolute and relative $\dot{V}O_{2\text{ peak}}$ were significantly greater ($P > 0.005$) in the high HRV group (17% and 20% respectively) compared to the low HRV group (6% and 1% respectively). The authors concluded that HRV was a significant predictor of response to exercise training in sedentary adults. It is of note that the term HRV is used in this case to represent the amplitude of heart rate variability from peak to trough during respiratory-mediated sinus arrhythmia. Prior to automated data processing techniques, this amplitude was

commonly reported in studies (Maciel *et al.* 1985; Maciel *et al.* 1986; De Meersman 1993). It was used as a simple measure of vagal modulation (Reiling and Seals 1988; De Meersman 1992). This simple time domain measure is not dissimilar to HF power measured from short-term recordings measured in the frequency domain.

Hedelin *et al.* (2001) studied changes in peripheral (peak torque) and central ($\dot{V}O_{2\text{max}}$) measures of response to exercise training in elite athletes. Amongst other associations, these authors found, that athletes who increased their $\dot{V}O_{2\text{max}}$ following training had significantly higher baseline values for HF(ln) than the equal number who showed a decrease in $\dot{V}O_{2\text{max}}$. In common with the design of the study by Boutcher and Stein, Hedelin *et al.* did not intend to show such an effect. The intention to treat analysis of these data was to show changes in HRV profiles due to training and relationships between change in peak torque, $\dot{V}O_{2\text{max}}$ and HRV. On this basis, as with the work of Pardo *et al.* no causative link between baseline HRV and response to training should be made. To date, only one study has set out with the express intention of demonstrating a causative link between an HRV measure at baseline and subject response to a standardised exercise training regimen. Hautala *et al.* (2003) trained 39 sedentary males six times per week for 8 weeks and assessed their change in $\dot{V}O_{2\text{peak}}$ over the course of the training period. When subjects were divided into quartiles based on response ($\Delta\dot{V}O_{2\text{peak}}$) there were no differences in baseline measures of: $\dot{V}O_{2\text{peak}}$, age, or BMI. Heart rate and heart rate variability measures were calculated from 24-hour Holter monitoring. Neither 24-hour, day time or night time HR were not different between response groups, all HRV measures (SDNN, LF, VLF, ULF) were similar except HF(ln). When calculated for the entire 24-hour period HF(ln) was significantly lower in the low response group (4th quartile) than the high response group. When calculated from ECG recorded from midnight to 6 AM the average-low response and the low response (3rd and 4th quartiles) were both significantly lower than the high response group.

9.4.3. *Linear regression analysis*

9.4.3.1. Predictors of MWT at baseline.

At baseline, $\dot{V}O_2$ peak was the only significant predictor of MWT. This finding is concordant with data from healthy individuals, and at first sight seems discordant with the assertion that IC limits exercise tolerance in the PAD patient. The fact that disease severity (ABI) was not predictive of MWT opposes the view of many previous data. Two reasons can be given to explain this finding.

Firstly, ABI may be a poor measure of muscle blood flow during exercise (Green 2002). Previous data have shown increased exercise tolerance due to training without change in ABI. Conversely, increases in ABI due to surgical intervention have been found to be unrelated to changes in pain free walking and exercise capacity (Regensteiner *et al.* 1993). It is not in doubt that IC limits walking in PAD patients, all patients in the present study reported leg pain during exercise. It may not, therefore, be the case that exercise induced IC does not impact on MWT but merely that in the present study a suitable measure of muscle blood flow was not made.

Secondly, it may be that $\dot{V}O_{2\text{ peak}}$ and ABI are in some way related or at least dependent. In the general population, $\dot{V}O_{2\text{ peak}}$ is dependent on levels of habitual physical activity. This is undoubtedly, also true in patients with PAD. The greater the level of physical activity the higher the $\dot{V}O_{2\text{ peak}}$ will be in each patient. In addition to this, walking economy may be higher for the same reasons. If, ABI and therefore, IC, have any impact on physical activity it is likely that they will be related to MWT. Although these measures were entered into the regression equation, none significantly predicted MWT. Collinearity diagnostics revealed that ABI was, to a degree, collinear with $\dot{V}O_{2\text{ peak}}$. It seems therefore, that the above

argument holds, insofar as $\dot{V}O_{2\text{ peak}}$ and ABI may be intrinsically linked via physical activity levels and that the former acts at least partially, as a surrogate for the latter in the present regression analysis.

9.4.3.2. Prediction of Δ MWT over 12 weeks of training.

From an experimental and clinical point of view the prediction of MWT is interesting and useful. From a clinical standpoint, factors that can predict change in MWT are of greater importance. When anthropometric, physiological and HRV measures were used to predict Δ MWT, $\dot{V}O_{2\text{ peak}}$ and HF(ln) were both significant independent predictors.

The above analysis was performed on the whole patient population, it is difficult to make causative conclusions based on such data as patients received different treatments. Therefore, an identical analysis was performed on the SU group only.

Separate analysis of the SU group data confirmed $\dot{V}O_{2\text{ peak}}$ and HF(ln) again, to be significant independent predictors of Δ MWT. It seems, therefore, that subject levels of peak oxygen utilising capacity may be important in determining response to rehabilitation. It is of note here that initial MWT was not a predictor of Δ MWT. In fact, no significant relationship between these variables was evident. This may suggest the some patients may have had lower walking economy at the beginning of the trial. As walking economy is known to improve with training (Gardner *et al.* 2002) these subjects may have shown large improvements in MWT. Alternatively, it could be that patients with initially higher $\dot{V}O_{2\text{ peak}}$ levels could better tolerate the treadmill walking exercise, performing longer walking bouts at higher intensities. This would result in greater gains for these patients. An initial $\dot{V}O_{2\text{ peak}}$ value above a certain threshold may have aided these patients in gaining greater benefit from the

training programme. Finally, it could be that patients with initially higher $\dot{V}O_{2\text{ peak}}$ values were simply those patients willing to 'push themselves' during the initial treadmill testing protocol. It follows that someone who puts in maximal effort at the initial test is likely to also try hard during the training programme. It has been demonstrated that walking programmes that encourage PAD patients to exercise at near maximal levels of pain produce the biggest gains in walking improvement (Gardner and Poehlman 1995). Further analysis of the exercise test data, especially calculations of change in walking economy and $\dot{V}O_{2\text{ peak}}$ values may provide more definite answers to the tentative explanations put forward here. Analysis of patterns of exercise (duration and number of stops per session), and effort (RPE and pain scale scores) may also provide further valuable information. These analyses are, however, beyond the scope of this chapter.

The fact that HF power, a marker of vagal modulation of the SA node is also an independent predictor of training response warrants further investigation. Again, only very tentative explanations regarding possible causative relationships between training response and HF can be made based on the present data due to the small sample size.

One possible explanation is that as HRV was measured at rest where vagal modulation predominates that HF, actually more closely represents the global autonomic control system. Such a system will allow greater exercise tolerance via acute adaptation (e.g. ability to raise heart rate) which may in turn facilitate higher intensity exercise and create enhanced chronic adaptation. Another explanation may be related to evidence of increased vagal modulation *per se*. Enhanced vagal modulation of the SA node will enhance acute exercise recovery and HF has been shown to be related to heart rate recovery in a number of populations. Faster recovery means shorter rest intervals between bouts of walking within training sessions, which would in theory, create an enhanced training response. Again further research into this area is required to support such a notion.

A final reason for the predictive power of HF may be that it is a marker of disease severity. Those patients with the most severe form of PAD are likely to be those who are most limited in their daily activity by PAD. These are likely to be the patients with lowest HF and $\dot{V}O_{2\text{ peak}}$ for that matter. Therefore, patients with higher HF power at baseline may have represented a subpopulation of PAD patients. However, only resting ABI was available as a measure of disease severity and given the shortcomings of this measure described above, little inference regarding this notion can be made here. However, prior to any attempt to utilise either $\dot{V}O_{2\text{ peak}}$ or HF as markers of potential training response, factors which determine levels of these two measures at baseline require further investigation.

Potential covariates in this population which could not be controlled for due to small subject numbers may include; pharmacological therapy such as beta blockade (Malfatto *et al.* 1998), antecedent related illnesses and medical interventions such as prior MI (Bonnemeier *et al.* 2000) or revascularising surgery (Tseng *et al.* 1996; Osterhues *et al.* 1998; Ozcan *et al.* 1999; Wennerblom *et al.* 2000) and any other accompanying illnesses such as diabetes (Malpas and Maling 1990). All of the above conditions or treatments were represented in the present PAD patient population and all are known to affect HRV in other populations. However, a much larger sample size would be necessary to make any comparison or inference about the effect each had on HRV and especially HF.

9.4.4. *Changes in HRV at equal absolute exercise intensity.*

Numerous pathological conditions are diagnosed routinely today by the use of exercise 'stress' testing. To determine if any change in the autonomic function of PAD patients was evident during exercise, HRV analysis was carried out in the SU group at weeks one, six and twelve of the exercise programme. This analysis was

carried out based on the assumption that exercise HRV may reveal changes in autonomic function despite no changes being evident at rest. This has been shown previously in healthy subjects of a similar age to the PAD patients studies here (Myslivecek *et al.* 2002).

Significant changes in RR interval and HF(ln) were evident. The increases in both these measures demonstrating a lower heart rate, accompanied as expected by increased vagal modulation. These findings represent a preservation of vagal tone during exercise. This has been shown previously in healthy populations. Levels of HF power are commonly higher during equal absolute intensity exercise in trained than untrained subjects (Gregoire *et al.* 1996) and may be increased by exercise training (Leicht *et al.* 2003; Leicht *et al.* 2003). It has previously been stated that preserved vagal modulation of the SA node during exercise may create a more electrically stable environment. On this basis, it has been argued that by demonstrating increased preservation of HF during exercise following a training intervention subjects also demonstrate a safer exercise HRV profile. Previously, this has only been shown in healthy subjects, who were not at risk of problematic cardiac electrical events. The low HRV values as well as attenuated exercise capacities, and historically poor prognosis for PAD patients places them in a high risk category for future cardiac event (Dormandy *et al.* 1999). The finding of increased HF at equal absolute exercise intensity may be of some importance. This is also the first time that increases in this measure have been demonstrated empirically in any clinical population.

The findings of an increase in LF:TP ratio (sympathetic marker) may seem somewhat contradictory. This is especially true when it is considered that while HF remained stable from week six to week twelve and LF:TP increased, there was actually a concomitant increase in RR interval.

This finding can be explained by paying attention to the nature of HRV recordings made here. First of all, it is not uncommon for both LF and HF spectral powers to increase post-training when measured at rest and during exercise (Leicht *et al.* 2003; Leicht *et al.* 2003). Such findings and the increase in LF:TP in the present study are due to the fact that the HRV measures (LF, HF and LF:TP) all measure modulation of the SA node. They are not measures of sympathetic or parasympathetic tone and do not measure mean firing rates of either branch of the ANS (Goldberger *et al.* 1994; Goldberger 1999). It therefore follows that if increased vagal modulation is present (as implied by increased HF) during exercise, then a greater level of sympathetic modulation will be required to elicit a similar HR response. What, it seems, is happening during exercise in the trained patients is that a preserved vagal modulation is maintained but that an increase in sympathetic modulation is also present. The latter may be necessary to facilitate adequate excitation of the SA node to maintain heart rate, cardiac output and therefore, exercise capacity.

Of interest when observing the changes in RR interval, HF and LF:TP are the differences in the time course of their adaptation. Initially there were significant increases in RR interval and HF (weeks one to six) with no change in LF:TP. Between weeks six and twelve, HF remained relatively constant while significant increases in LF:TP were observed. The meaning of and reasons for the different adaptive behaviours of these measures also requires further investigation.

9.5. Conclusions.

The findings of the present study agree with previous data showing significant increases in MWT in PAD patients undertaking supervised treadmill walking exercise. Increased exercise capacity was not accompanied by increased $\dot{V}O_{2\text{ peak}}$ during treadmill testing or measures of HRV made at rest. These findings together suggest that the training stimulus provided by treadmill walking exercise was insufficient to produce central physiological and autonomic adaptation. One reason

for this may have been the discontinuous nature of the walking exercise performed. This is a feature common to all studies in PAD patients.

Heart rate variability measures designed for use during exercise showed significant alterations in levels of sympathetic and parasympathetic modulation resulting in a net overall decrease in exercise heart rate. It seems, therefore, that HRV measured during exercise may be able to illustrate changes in autonomic function which cannot be observed when the nervous system is assessed at rest.

In common with findings in healthy subjects and other clinical populations, it was shown that responders and non-responders to exercise training differed from each other at baseline in terms of HF spectral power. The fact that baseline HF was higher in those who consequently showed the biggest increases in maximal walk time is of particular interest, especially when taken with the fact that the baseline HF was a significant independent predictor of Δ MWT in the SU group. The nature of the relationship between training response and baseline HF requires further investigation with a larger subject population.

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CHAPTER 10. CHANGES IN RESTING HEART RATE VARIABILITY IN PATIENTS UNDERTAKING EIGHT WEEKS OF CARDIAC REHABILITATION.

Abstract.

Following myocardial infarction, alterations in the activity of the autonomic nervous system occur. These are characterised by a dominating sympathetic contribution to autonomic balance reflected in an overall reduction in heart rate variability (HRV). The aim of this study was therefore, to evaluate the effects of cardiac rehabilitation (CR) on autonomic function by heart rate variability.

Thirty-eight patients (21 males and 17 females, mean age 56 ± 8 years) patients underwent 5 min, resting ECG recording at CR entry and exit assessments. ECG data were automatically filtered and a time series of normal to normal RR intervals created. The mean (NN) and the SD of normal to normal intervals (SDNN) were created. A fast Fourier transform was then applied to calculate the power spectral density of the NN intervals. Total spectral power (TP, 0.04 – 0.40 Hz) was divided according to recommended guidelines into the vagally mediated high (HF, 0.15 – 0.40 Hz) and the mixed, sympathetic and vagal low frequency components of the power spectrum (LF, 0.04 – 0.15 Hz). The ratio of these measures (LF:HF) was calculated as a measure of sympathovagal interaction. Values were log transformed and compared using repeated measures ANOVA, *t*-tests and analysis of covariance where appropriate.

Global (SDNN) and spectral measures (LF, HF) were all increased following CR ($P < 0.05$) using both methods of statistical analysis. There was a trend toward increased NN interval but no change in LF:HF.

HRV measures (SDNN, LF and HF) are risk factors for future cardiac event following myocardial infarction. In the present study, these measures of autonomic

modulation were all increased. An increase in HRV provides protection against cardiac arrhythmia, demonstrating that CR is an effective therapeutic intervention.

10.1. Introduction.

Following myocardial infarction (MI), alterations in the activity of the autonomic nervous system (ANS) occur, characterised by a dominating sympathetic contribution to autonomic balance. In post-MI patients, this is reflected by a reduction in overall heart rate variability (HRV), particularly in measures associated with vagal modulation (Bigger *et al.* 1992). There are substantial time and frequency domain data from 24-hour Holter measurements of HRV to describe the autonomic balance of post-MI patients. These data suggest that severe derangements are evident after a myocardial infarction (Bigger *et al.* 1992; Bigger *et al.* 1995). There is also evidence that, to a certain degree, these derangements recover spontaneously in the months following MI (Bigger *et al.* 1991). Despite this spontaneous recovery, values remain well below that of the normal population (Bigger *et al.* 1995). Perhaps more importantly, reduced 24-hour HRV values after MI are strong, independent predictors of future cardiac event and death (Malik *et al.* 1989; Malik and Camm 1990; Bigger *et al.* 1991; Breithardt *et al.* 1995; Quintana *et al.* 1997; Klingenhoben *et al.* 1998; Liu *et al.* 2003). Due to its association with poor prognosis, HRV has been proposed as a potential therapeutic target in numerous populations including post-MI patients (Routledge *et al.* 2002).

In previous chapters, the effects of chronic exercise on HRV measures were discussed. Recent reviews on this topic have drawn the common conclusion that HRV may be increased following exercise training. Commonly, global increases in HRV are accompanied by a shift in sympathovagal balance toward increased vagal modulation at rest.

10.1.1. *Post-myocardial infarction changes in HRV due to rehabilitation – a brief review of the literature*

Data concerning changes in HRV measures in response to cardiovascular rehabilitation are relatively common. However, the majority of these HRV data have been generated from 24-hour, ambulatory ECG measurements. What follows is a brief review of the literature concerning the effect of post-MI CR on HRV. Unless otherwise stated, all data are drawn from 24-hour ambulatory ECG recordings.

Mazuero *et al.* (1992) found HRV values close to those reported for healthy subjects 4 - 6 weeks after anterior wall MI in $n = 38$ patients. Patients were randomised into exercise or control groups and retested six months later. An increase in standard deviation of normal-to-normal intervals (SDNN) and a shift toward resting sympathetic predominance (increased LF:HF ratio) were found. The effect sizes for the changes observed were small, and subjects showed heterogeneous responses. The authors concluded that spontaneous return to baseline autonomic balance was probably responsible for changes and that any conclusions made on the basis of this study should be cautious.

Leitch *et al.* (1997) found a short (6-week) exercise programme was effective in increasing exercise capacity but not HRV measures when only hospital-based participants ($n = 26$) were included in the statistical analysis. When home-based participants ($n = 23$) were included there were small but significant changes in global HRV, (SDNN). There were no differences between HRV changes in the home-based (control) subject group and the experimental group. These authors also conceded that these data may indicate a spontaneous return toward normal HRV values and suggested that as only subjects with uncomplicated MI were included, results from this study should not be generalised beyond this population. In an elderly cohort on a residential rehabilitation programme, Stahle *et al.* (1999) found increased global 24-hour HRV (SDNN) due mainly to increased day-time variation.

Increases in long-term variation of HR, measured by the standard deviation of the means of all normal-to-normal 5-min periods of measured time period (SDANN) suggest this may be due to increased ambulatory activity in this group. However, the authors fail to recognise this and suggest a significant increase in HRV due to their exercise intervention. The small effect sizes for SDNN ($d = 0.32$) and SDANN ($d = 0.20$) are the only two statistically significant differences from a total of 38 longitudinal comparisons made. No statistical adjustment for multiple comparisons was made by the authors (e.g. Bonferroni), therefore any interpretation of these data should be made with extreme caution.

Malfatto *et al.* (1998) combined exercise and β -blocker therapy and found an additive effect compared with either therapy alone in reversing derangement of autonomic profile after first MI. These data showed a blunted sympathetic and increased parasympathetic profile in patients receiving β -blockers four weeks after MI. Further increases and decreases in parasympathetic and sympathetic modulation respectively were found after 12 weeks, only in the subjects who also exercised. Those subjects receiving β -blocker therapy alone showed no further improvements in autonomic profile. Unfortunately, no follow-up data on factors such as reinfarction or survival rates are available. Similarly, Pardo *et al.* (2000) found small but statistically significant increases in vagal modulation measured by high frequency power (HF) in patients after 12 weeks of rehabilitative exercise. No pre- and post-test differences were found for any other HRV measures, although differences between changes in HRV measures were evident in a subgroup analysis based on patient gains in exercise capacity following rehabilitation.

Tygesen *et al.* (2001) found increases in exercise capacity were related to global measures of HRV in patients following MI and coronary artery bypass grafting (CABG). Frequent training ($6 \cdot d^{-1} \cdot wk^{-1}$) increased exercise capacity and HRV more than less frequent ($2 \cdot d^{-1} \cdot wk^{-1}$) training. In the former condition, changes in HRV and exercise capacity remained evident a year after completion of training. La Rovere *et al.* (1992) found evidence of sympathetic overactivity in post-MI patients. After four

weeks of either hospital based training or no exercise neither group showed significant alterations in autonomic profile at rest. However, during passive tilt, the trained group showed enhanced parasympathetic withdrawal and sympathetic activation. The authors concluded that training had induced restoration of autonomic reactivity to near-normal values.

Using an 8-week training period and short-term analysis of HRV, Duru *et al.* (2000) found exercise capacity and time domain measures of HRV were increased, but that spectral measures of HRV were unaffected. Control subjects showed a significant decrease in global HRV measures after 12 months. The authors concluded that a shift in sympathovagal balance toward sympathetic predominance was evident in the untrained group and that training had attenuated such a shift in the trained subjects.

The present study and those cited above all recognise the potential confounding effects of cardioactive pharmacologic therapies. Most studies have measured HRV under 'normal' pharmacologic conditions. Under current guidelines, such pharmacotherapy usually includes β -blockers, ACE inhibitors and other cardioactive drugs (Joliffe *et al.* 2001). Whereas it is realised that these drugs may affect HRV, it should also be recognised that ethical problems issues surrounding withholding recommended pharmacotherapy would preclude much research in this area.

10.1.2. Chapter aims and justification.

The main aim of this chapter is to determine whether short-term, resting measures of HRV can be improved by cardiac rehabilitation as offered in a British district general hospital. The results and conclusions of this study, will differ distinctly from those reviewed above due to several factors.

10.1.2.1. Time to entry into cardiac rehabilitation.

In the studies mentioned above, time to rehabilitation ranges from five days to

‘within two months’ (See Table 10-1). By making some simple assumptions such as ‘within second month’ being approximately six weeks the mean time from event or surgery to baseline assessment is approximately 37 ± 11 days.

Table 10-1 Reported time from myocardial infarction or surgery to baseline assessment for entry into cardiac rehabilitation.

Reported Time	Study	Country of Study
28 days	La Rovere <i>et al.</i> (1992)	Italy
Within second month	Mazzuero <i>et al.</i> (1992)	Italy
5 – 7 days	Leitch <i>et al.</i> (1997)	Australia
1 month	Malfatto <i>et al.</i> (1998)	Italy
6 weeks	Stahle <i>et al.</i> (1999)	Sweden
14 – 16 days	Iellamo <i>et al.</i> (2000)	Italy
36 (14) days	Duru <i>et al.</i> (2000)	Sweden
40 (22) days	Pardo <i>et al.</i> (2000)	USA
One month	Tygesen <i>et al.</i> (2001)	USA
Four weeks.	Lucini <i>et al.</i> (2002)	USA

Due to economic circumstance and limited resources, initial CR assessment is often longer than four weeks in the United Kingdom. Although this is in many ways problematic for both patients and staff, it does create a unique scientific perspective by which to examine the effects of CR on HRV after surgery or MI. In a landmark study (Bigger *et al.* 1991) it was shown that in 24-hour HRV values of post-MI patients, initially only 33– 50% of those recorded in healthy controls showed significant spontaneous recovery in the first three months following MI. There were, however, no further increases at either 6 or 12 months. From these findings it can be seen that the mean (\pm SD) study baseline assessment period in all but a few subjects in a single study (Iellamo *et al.* 2000) lie well within this boundary. Therefore, it is of interest to the scientist and rehabilitation practitioner alike to determine whether HRV, an important risk factor for future cardiac events in this population can be further increased beyond the time scale of this known period of spontaneous autonomic recovery.

10.1.3. *The importance of control groups and appropriate statistical analyses.*

The majority of the studies employ some form of control group who either undertake home based activity (Leitch *et al.* 1997) or no exercise (Duru *et al.* 2000). Where control groups have been used, a statistical issue is raised. The question of whether a mixed analysis (group x time) with *post hoc* comparisons is used or whether a simple comparison of Δ HRV between experimental and control groups becomes important. For instance, Duru *et al.* claim to have used a 2 x 4 mixed analysis of variance (mixed ANOVA) to observe differences in HRV between an exercise group and controls (two groups) at baseline, one, two and 12 months (four time points.) They give no account of any proposed *post hoc* analyses. Results of this study are then strewn with *P* values relating to change in heart rate ($P < 0.01$), pNN50 ($P < 0.05$) and SDNN ($P = 0.06$). No information as to how these values were created or to what type of inferential statistic they relate is given. There also seem to be disparities in the baseline HRV characteristics of the two groups, particularly in frequency domain measures. For instance, normalised low frequency power (LFnu) in the experimental group (20.0 ± 10.1) greatly exceeded that in the control group (14.2 ± 4.1). Similarly, LF:HF in the experimental group (2.7 ± 2.9) was much greater than that of controls (1.9 ± 2.0). It would be foolhardy to assume that subject baseline characteristics do not influence response to rehabilitation especially in light of published data that suggest this to be the case (Malfatto *et al.* 2000). Other examples of poor description of data analysis methods include stating simply that 'Student's *t*-test were used' (Tygesen *et al.* 2001). This does not allow the reader to determine whether these were carried out between experimental and control group mean values pre- and post-rehabilitation or on change in measures. This study (Tygesen *et al.* 2001) also reports a very large number of individual comparisons with no mention or employment of a correction factor for alpha.

Based on previous findings (Bigger *et al.* 1991), it is unlikely that any spontaneous autonomic recovery will occur in the present study. This makes the employment of a

control group less important. It is of interest to note that none of the studies cited thus far have been based in the United Kingdom. Ethical approval committees in the UK for example, would not permit the randomisation of cardiac rehabilitation patients into control groups (Tan *et al.* 2003) as is clearly allowed in Sweden and the United States. Therefore, in certain cases where a control group has not been used (Pardo *et al.* 2000; Tygesen *et al.* 2001) such actions may be justified on an ethical basis. Some studies have used opportunistic sampling methods for subject group allocation, whereby those patients who decline their place in the rehabilitation programme are used as controls (Lucini *et al.* 2002). Other studies use no control group (La Rovere *et al.* 1992; Pardo *et al.* 2000). Both these methods are problematic. The latter makes any conclusions regarding causative effects of rehabilitation on change in HRV difficult. The process of self-selection in the former method may lead to significant between-group differences at baseline. In the study of Lucini *et al.* (2002) for example, controls were significantly younger, heavier and had worse cholesterol profiles than the experimental groups.

In other studies, control groups have been used which are clearly different to intervention groups on certain HRV characteristics at baseline. Authors have failed to provide any statistical tests of these differences on two occasions (Mazzuero *et al.* 1992; Malfatto *et al.* 1998). Additionally, only one study has evaluated the strength of the relationship between baseline scores and Δ HRV (Malfatto *et al.* 2000); this is also a recommended step in pre-/post-test designs with controls that differ at baseline (Altman 2003).

It is of interest to note that in the single study in the literature which has carried out an independent test between Δ HRV for an intervention group and a control group, no differences in the change of any HRV measures were found (Leitch *et al.* 1997). Within this study, changes in HRV were of comparable direction and magnitude to those reported as significant in other investigations using paired analyses, but concomitant increases in HRV measures in the control group negated these findings.

Due to ethical restrictions and the increased time to onset of rehabilitation, the methods for this chapter will apply a novel approach, using age matched controls from the vascular outpatient clinic with conditions which are not currently referred for rehabilitation. In this way, the ethical concern of withholding therapy known to be efficacious is removed. Additionally, a reasonably well matched group of subjects can be used to monitor any time-dependent changes in HRV measures. With this approach, a statistical analysis similar to that employed by Leitch *et al.* will also be used.

10.1.3.1. Baseline resting HRV measures as indicators of adaptation to training.

In addition to monitoring any changes in resting HRV due to rehabilitation in the CR group, a further aim of this chapter is to investigate the potential of certain HRV measures to predict responses to exercise training.

Knowledge of a patient's autonomic profile at entry to CR may be of use for a number of reasons. As well as providing information regarding cardiac risk, certain measures of HRV may provide additional information regarding appropriate exercise prescription. Firstly, patients with evidence of preserved vagal modulation may have a lower likelihood of a cardiac event during exercise at any given absolute workload. On this basis, such patients may be encouraged to exercise at greater relative and absolute exercise intensities, dependent on initial exercise capacity.

A further application of resting HRV measures, may be as an aid to exercise prescription and rehabilitation goal setting. Data from healthy subjects have shown that certain measures of HRV are predictive of training response (Boutcher and Stein 1995; Hedelin *et al.* 2000; Hautala *et al.* 2003). Some similar data from patients with peripheral vascular disease are presented in the previous chapter.

Boutcher and Stein (1995) studied the effect of exercise training on heart rate variability (HRV) and improvements in peak oxygen consumption ($\dot{V}O_{2\text{ peak}}$) in sedentary middle-aged men. The HF power, absolute $\dot{V}O_{2\text{ peak}}$ and relative $\dot{V}O_{2\text{ peak}}$ of trained subjects ($n = 19$) and controls ($n = 15$) were assessed before and after a 24-session training programme. Absolute and relative $\dot{V}O_{2\text{ peak}}$ increased ($P < 0.005$) for the training group (12% and 11% respectively); there were no changes in HF. This was despite a significant reduction in resting heart rate. However, when the trained subjects were divided according to high ($n = 5$) and low ($n = 5$) HF at baseline it was found that changes in $\dot{V}O_{2\text{ peak}}$ were significantly greater ($P < 0.005$) in the high HF group for both absolute (17%) and relative (20%) values. Changes in the low HF group were 6% and 1% for these measures respectively. There were no group differences in mean age, pre-training $\dot{V}O_{2\text{ peak}}$, or resting heart rate in the low and high HRV groups. The authors concluded that individual differences in baseline HRV could be associated with the response of $\dot{V}O_{2\text{ peak}}$ to aerobic training. This study demonstrated differences in training response in groups divided by HRV characteristics. However, no relationship between HRV and training response was demonstrated. Later studies examining the prognostic significance of HRV in relation to training response have, however, succeeded in demonstrating this.

In the first of two studies from the same laboratory, Hedelin *et al.* (2001) studied a mixed (eight females and nine males) group of elite cross-country skiers during their normal training regimen. The start and end points of the data collection protocol were in the off-season and just prior to the start of competition respectively. Heart rate variability was measured using RR intervals derived from short-term 5-min measured in the supine position and during tilt a manoeuvre. Central performance ($\dot{V}O_{2\text{ max}}$) and peripheral performance (peak isokinetic torque and time to peak torque) were used as indicators of training effect. The main methodological weakness with this type of opportunistic research is a lack of control concerning the independent variables involved. Specifically, training differed considerably between subjects in terms of type, intensity duration and volume. This resulted in some

subjects showing significant gains in central and peripheral performance while others showed no change or a decline inferring a negative training effect. When subjects were divided according to whether increases or decreases in $\dot{V}O_{2\max}$ had occurred, the authors found significant differences in baseline HF between groups. Specifically, those subjects with higher levels of HF at baseline demonstrated significantly ($P < 0.05$) greater increases in $\dot{V}O_{2\max}$. Power in the HF band was unrelated to improvements in peripheral (strength) performance. However, resting LF power (ms^2) measured at baseline was negatively related to change in maximal lactate accumulation. Response of LFnu to tilt (ΔLFnu) was negatively related to improvements in peak torque and time to peak torque measured isokinetically. That is, performance in subjects highly reactive to orthostatic challenge (large sympathetic response to tilt) either became worse or improved less than those subjects with a small response.

Unfortunately, this study has a weakness not addressed by the authors. A wide range of values for HF, LFnu and ΔLFnu were found at baseline and training response was heterogeneous. The subjects used were elite athletes and it seems likely that some subjects may have been overtrained or at least overreached at baseline. These conditions are associated with increased sympathetic predominance, which is known to manifest itself as a reduced variability in heart period, particularly those oscillations in the HF spectral band (Blaber *et al.* 1996; Scalvini *et al.* 1998; Uusitalo *et al.* 2000; Bosquet *et al.* 2003). Additionally, subjects who demonstrate cardiac sympathetic predominance at rest typically show reduced reactivity to orthostatic challenges (Blaber *et al.* 1996; Scalvini *et al.* 1998; Mourot *et al.* 2004). If those subjects who decreased their performance due to training were approaching or within a state of overtraining they would display low ΔLF . Healthy subjects would show a more favourable autonomic profile and be able to train in a manner that elicited increases in central and peripheral performance. Overtrained subjects would also be unlikely to show improvements due to training and more likely to show decrements in performance as indeed around half the subjects in this study did.

Hautala *et al.* (2003) trained previously sedentary males six days a week at an intensity of 70-80% $\dot{V}O_{2\text{ peak}}$ for 8 weeks and found that those subjects with higher levels of HF power at baseline demonstrated greater increases in $\dot{V}O_{2\text{ peak}}$. The correlation was strongest between night-time HF and runtime ($r = 0.52$), 24-hour HF and daytime HF also correlated significantly. Increases in performance were negatively associated with subject age ($r = -0.48$) and when this was controlled for, a significant relationship between increased run time and nocturnal measures of HF existed. The authors concluded that baseline cardiovascular autonomic function is an important indicator of improvement in aerobic performance due to training. Maximal oxygen consumption, age and HF were unrelated at rest, implying that HF power (especially when measured at rest) may be a true independent predictor of adaptation to training.

10.1.3.2. The application of findings to clinical populations.

The data given above relate only to healthy subjects and the generalisation of such findings to clinical populations requires caution. There are, however, data to suggest a link between baseline autonomic function and training response of the autonomic nervous system in post-MI patients (Carunchio *et al.* 2000; Malfatto *et al.* 2000). Carunchio *et al.* (2000) studied 122 patients undergoing 8 weeks of cardiac rehabilitation following MI. Of these patients, 58 were randomised into an exercise group, the remainder made up a group of control subjects. All subjects underwent 24-hour monitoring at baseline. Of the exercise group, analysable data were available in 30 subjects after eight weeks of training. It was found that pNN50, HF and LF:HF ratio were all favourably altered following rehabilitation, indicating increased cardiac vagal modulation. A positive correlation ($r = 0.48$) was found between LF:HF ratio at baseline and change in this measure after rehabilitation. This was found to be independent of ejection fraction, changes in creatine phosphokinase and location of infarction. These authors concluded that sympathovagal balance at baseline may act as an indicator of adaptability of autonomic function in this population. Malfatto *et al.* (2000) also found a

significant, positive relationship between baseline LF:HF and Δ LF:HF due to rehabilitation.

To summarise, it seems that in healthy populations, high levels of vagal modulation (measured by HF) may indicate increased trainability in terms of exercise performance and physiological adaptation to training. In post-MI patients, higher vagal modulation (LF:HF ratio) may indicate an even greater potential for the autonomic nervous system to adapt.

10.1.4. Hypotheses and structure of consequent sections.

The results, and consequent discussion of findings within this chapter will therefore, be divided into three distinct parts to investigate three distinct hypotheses.

- i. Longitudinal data will be used to test the hypothesis 'Eight weeks of cardiac rehabilitation can favourably increase measures of HRV.'
- ii. Baseline measures will be used to test the hypothesis 'High frequency spectral power can independently predict improvements in shuttle-walking test performance.'
- iii. Longitudinal data will be used to test the hypothesis: 'LF:HF ratio predicts changes in autonomic balance as measured by change in LF:HF.'

10.2. Methods.

Subject sample size was estimated based on the results of previous studies and from effect sizes calculated in a small meta-analysis. A methodological description of the meta-analysis approach used can be found in Chapter eight and the analysis itself can be found in appendix four.

10.2.1. Subjects.

Subjects were 21 males and 17 female patients referred for CR at the Hillingdon hospital between September 2003 – January 2005. Patients were recruited by including an information sheet and written consent form in the invitation to CR sent to all patients. All procedures were approved by the local research ethics committee and conformed to the guideline presented in the declaration of Helsinki (World Medical Association) for research with human subjects. Criteria for exclusion were coexisting valvular disease, contraindications to exercise such as frequent atrial or ventricular premature beats, orthopaedic limitations that prevented exercise testing and any other serious post-operative complaints or illnesses.

10.2.1.1. Control group.

Twenty-three control subjects were recruited from the vascular outpatient clinic at the same hospital. The descriptive characteristics of this group can be found in table 10-2. All patients had a history of cardiac disease of ischaemic origin. The mean ages of the two patient groups were matched, but no attempt to match for disease aetiology was made. Heart rate variability analysis was carried out in a similar manner in both groups, although the controls did not attend a cardiac rehabilitation assessment or complete the shuttle-walking test.

10.2.2. Protocol.

All initial measurements were made during the entry consultation to CR. Patients completed the standard hospital anxiety and depression inventory (HAD). This was followed by a brief interview with the specialist cardiac rehabilitation Sister. During this time details on family history, activity levels, and general lifestyle and quality of life values were assessed.

Patients were then fitted with a Polar™ S810 heart rate monitor (Polar Electro OY, Kempele, Finland) and if a significant number of premature atrial or ventricular beats were expected based on their assessment; a Cardionetics Holter monitor was also fitted. Patients then laid supine on a plinth with head supported on a pillow and were instructed to lay quietly. After two minutes of relaxation the RR interval recording began and continued for five minutes. Respiratory rate was not controlled during the measurement. After five minutes patients were asked to stand slowly, they were then given oral instructions regarding the completion of the modified shuttle-walking test. This activity took approximately four minutes, in this time RR interval data were recorded continuously.

10.2.2.1. Modified shuttle-walking test procedure.

Subjects walked on a gymnasium floor between two cones, ten metres apart in time to an audible beep on a tape recorder. Subjects were instructed to keep up with the pace but not to exceed it. The test started at a speed of $0.5 \text{ m}\cdot\text{sec}^{-1}$, at which the subject completed three shuttles per minute, the speed of walking was then increased so that an extra shuttle was completed during the same time period during each subsequent stage to maximum velocity of $2.4 \text{ m}\cdot\text{sec}^{-1}$. Exercise was terminated when the subject reached volitional exhaustion, a heart rate within 10% of estimated HR max ($220 - \text{age}$) or when they failed to maintain a pace sufficient to reach the cone in time on three successive occasions.

Following test termination patient, the walked two further shuttles at a self-selected pace and then sat down. Blood pressure was taken while seated and the patient was instructed to relax and breathe normally. Patients remained seated until heart rate had returned to within ten beats of their pre-exercise value.

10.2.2.2. Measurements of heart rate response.

Patient heart rate was recorded at the end of each stage of the test. Heart rate recovery was recorded as the difference between maximal exercise heart rate and that recorded at 1-min after exercise cessation. The changes in heart rate in the first minute after the subject assumed the seated position were also recorded.

10.2.2.3. Measurements of heart rate variability.

Heart rate variability measures were derived from the 5-min of stationary ECG obtained at rest using the Polar monitor, in accordance with current recommendations (Taskforce 1996).

The Polar Precision Performance SW 3.02 software (Polar Electro OY, Kempele, Finland), contains an automatic, RR interval filtering and interpolation algorithm. Prior to extraction of any segments the entire time series was error corrected using a medium filter power and minimum beat protection zone of 6 BPM. The interpolation of beats via this method has only minor effects on spectral measures of HRV measured from stationary tachograms (Jurca *et al.* 2004) in which <15% of beats are rejected. Further technical details of the beat filtering and interpolation algorithms have been presented previously (Huikuri *et al.* 1992; Huikuri *et al.* 1996).

After filtering all RR interval time series were stored as .txt files on the hard drive of a password protected computer for later analysis.

10.2.2.4. Heart rate variability analysis.

Filtered RR interval data were imported into the Software for advanced HRV analysis (Universtiy of Kuopio, Finland). This software has been developed in accordance with published recommendations (Taskforce 1996) and the findings of two validation studies using RR interval data collected using a Polar S810 or

analogous model (Ruha *et al.* 1997) and consequently analysed by the programme have been published (Niskanen *et al.* 2004).

Within the HRV analysis software, the filtered data were detrended using the smoothness priors method (Gersh 1991), which behaves like a time varying finite impulse response high-pass filter. It is easy to apply to different occasions as it only has a single adjustable parameter.

Using the smoothness priors method to detrend the time series obtained during short-term measurements of HRV effectively removes the large very low frequency (VLF) spectral component derived by the fast Fourier Transformation method of obtaining a power spectrum. Although this method of detrending removes the VLF component, power within the frequency bands of interest (LF and HF) remains largely unaffected (Niskanen *et al.* 2004). Using this method it is possible to obtain comparable measures of LF and HF power under a number of different physiological conditions (supine, standing and exercise) and to compare the absolute, and relative distributions of spectral power within these bandwidths.

10.2.3. *Heart rate variability measurements and statistical analysis for each comparison.*

10.2.3.1. Changes in autonomic function due to cardiac rehabilitation.

To compare resting pre- and post-CR measurements taken from the 5-min, stationary tachogram recorded in the supine position, SDNN and RMSSD were calculated in the time domain. In the frequency domain, low frequency power (LF, 0.04-0.15 Hz), high frequency power (HF, 0.15-0.40 Hz), were measured in both in raw and normalised units (HFnu and LFnu). Additionally the LF:HF ratio was calculated and used as a measure of sympathovagal balance (Pagani *et al.* 1986). Statistical analysis was only carried out on: LF, HF and LF:HF ratio in the frequency domain, SDNN, and rMSSD in the time domain. These measures have been investigated

previously in healthy and clinical populations and are relatively well defined. Making *a priori* selections of measures for analysis reduces the total number of comparisons made. This avoids problems similar to those mentioned in relation to previous studies (Stahle *et al.* 1999). Data were first transformed, if necessary, to allow parametric analysis. Effectiveness of the CR programme on HRV measures was assessed by an independent *t*-test of pre- and post-test differences between the experimental and control groups. In the case of significant differences between the CT and the CR group in HRV measures at baseline, initial HRV values were controlled for using analysis of covariance (ANCOVA). The assumptions of homogeneity of variances and homogeneity of regression were both checked prior to the use of this test.

10.2.3.2. High frequency spectral power as a predictor of change in shuttle walking test performance.

Baseline high frequency spectral power (HF) was derived from the five minute tachogram, as described above and natural logarithms of this measure were taken to give HF(ln). The relationship between HF(ln) and change in shuttle-walking test performance measured as a percentage (Δ SWT%) and measured in metres (Δ SWTm) were assessed using a Pearson's product moment correlation coefficient. Other potential predictors were also assessed by correlation analysis. Those showing significant or close-to significant relationships with Δ SWTm were entered into a stepwise regression analysis.

10.2.3.3. The LF:HF ratio as a predictor of ANS adaptation.

The relationship between initial values of autonomic balance and changes in these values was assessed similarly to that described above but using initial LF:HF and Δ LF:HF. This analysis was performed only on data from the experimental group.

10.3. Results.

10.3.1. Patient baseline descriptive characteristics.

Table 10-2 shows the baseline descriptive characteristics of the cardiac rehabilitation (CR) and the control (CT) groups. The two groups were broadly similar in terms of age and sex distributions and were of similar weights. The CT group contained a lower proportion of diabetics than the CR group and as expected, the disease states and reasons for attending the outpatients clinic or rehabilitation programme were dissimilar between the groups. Reasons for not using a more exactly matched control group and the possible bearing this may have had on results of this study will be discussed later.

Table 10-2 Descriptive characteristics of cardiac rehabilitation and control group subjects at baseline.

	Cardiac Rehabilitation Group (n = 38)	Control Group (n = 23)
(Age years)	65.6 (11.6)	64.9 (9.0)
Sex	Males 21 (57%) Females 17 (43%)	Males 14 (61%) Females 9 (39%)
Mass (kg)	83.5 (20.2)	78.7 (20)
Diabetic	Yes n = 6 (17%) No N = 30 (83%)	Yes n = 1 (4%) No n = 22 (96%)
Disease state or reason for CR (%)	MI n = 17 (47%) Surgical procedure n = 19 (53%) MI and no procedure n = 11 (31%) MI with procedure n = 6 (16%)	Ischaemic cardiovascular disease n = 23 (100%) Previous MI (> 1year) n = 2 (8%) Previous cardiac surgical procedure (>1 year) N = 7 (31%)
Time from procedure or event (weeks)	16.5 (11.3)	N/A
Time to retest (weeks)	9.8 (1.9)	10.2 (3.8)
β-Blockers	Yes 23 (64%) No 13 (36%)	n = Yes 18 (64%) No n = 5 (36%)
Statin	Yes 6 30 (83%) No 6 (17%)	Yes n = 14 (61%) No n = 9 (39%)
Calcium channel antagonist	Yes n = 17 (45%) No n = 19 (55%)	Yes n = 6 (26%) No n = 17 (74%)
Aspirin	Yes n = 29 (83%) No n = 6 (17%)	Yes n = 12 (52%) No n = 11 (48%)
Other	Yes n = 6 (17%) No n = 29 (83%)	Yes n = 15 (64%) No n = 8 (36%)

All values are mean (\pm SD) for continuous measures and number of cases (percent) for categorical measures.

Table 10-3 shows changes in the standard physiological and psychological measurements taken at entry into the cardiac rehabilitation programme (risk analysis) and again at each patient's exit assessment. There were statistically

significant increases in 10 m shuttle-walking test performance and maximal exercise heart rate ($P < 0.05$), but not heart rate recovery. Other physiological variables such as blood pressure and subject mass also remained unchanged. Both measured psychological parameters (Anxiety and Depression) were significantly reduced post-rehabilitation.

Table 10-3 Physiological and psychological effects of cardiac rehabilitation.

	Pre-rehabilitation	Post-rehabilitation.	Significance.
Shuttle test score (m)	488 ± 214	597 ± 235	P = 0.001
Maximal heart rate (bpm)	119 ± 21	128 ± 27	P = 0.037
Heart rate recovery (bpm)	24.2 ± 12	30.0 ± 24	P = 0.148
SBP (mm/hg)	133 ± 19	134 ± 19	P = 0.808
DBP (mm/hg)	76 ± 7	78 ± 12	P = 0.309
Weight (kg)	83.5 ± 20	83 ± 20	P = 0.251
Anxiety	6.5 ± 3.9	4.6 ± 3.8	P = 0.001
Depression	5.6 ± 2.4	4.4 ± 3.5	P = 0.019

SBP – Systolic blood pressure, DBP – diastolic blood pressure, Anxiety and Depression are in arbitrary units measured by the hospital anxiety and depression inventory (HAD).

Table 10-4 Change in HRV measures of heart rate variability for cardiac rehabilitation and control groups.

HRV Measure	Δ HRV CR. Mean (C.I.)	Δ HRV CT. Mean (C.I.)	F – value for ANOVA or ANCOVA†	P-value for ANOVA or ANCOVA†	Significance of baseline measure as covariate
LF (ln)	0.586* (0.264 – 0.908)	-0.140 (-0.577 – 0.296)	6.433†	0.014†	0.001
HF (ln)	0.464 (0.137 – 0.790)	0.054 (-0.485 – 0.381)	3.388	0.071	0.189
LF:HF	-0.102* (-0.282 – 0.007)	0.121 (-0.116 – 0.358)	2.225†	0.142†	0.001
SDNN (ms)	6.785* (2.831 – 10.73)	-1.853 (-0.723 – 3.516)	4.698†	0.015†	0.035
RMSSD (ms)	7.035 (3.319 – 10.75)	-0.210 (-5.143 – 4.724)	4.656	0.035	0.344
RR (ms)	52.46* (17.97 – 87.05)	52.46 (-100.9 – 7.074)	12.48†	0.001†	0.001

* Indicates use of mean and C.I. adjusted for baseline values. † Indicates use of ANCOVA to adjust for baseline values.

10.3.2. *Change in heart rate variability measures due to cardiac rehabilitation.*

Table 10-4 shows the change in all HR and HRV measures over time in the CR and CT groups. Due to known associations between baseline HRV measurements and alterations, where appropriate, the baseline measure has been used as a covariate to control for its possible confounding effects. Where no relationship ($P > 0.05$ for ANCOVA covariate value) was found, ANOVA between Δ HRV measures was performed (HF and RMSSD). All other measures (LF, LF:HF, SDNN, RR) were analysed by ANCOVA.

All HRV measures displayed favourable shifts in their values in the CR group and none were evident in the CT group. The differences between change in HRV measures in CR was statistically significant for LF, SDNN, RMSSD and RR interval, changes in HF and LF:HF were not significantly greater in CR than in CT.

With the exception of LF:HF, the confidence intervals for Δ HRV in the CR group showed a relative homogeneity of response to the intervention. All C.I. s are positive for the remaining measures. In contrast the C.I. s for the CT group were much larger and contained both positive and negative values for Δ HRV.

10.3.3. *High frequency power as a predictor of change in shuttle walking test performance.*

To determine the capacity of HF(ln) to predict change in walking, Pearson's product moment correlation coefficients for baseline physiological variables and Δ SWT were calculated. In this instance Δ SWT was defined as the % change in total walking distance shown by each patient (Δ SWT%). Due to a significant positive skew these data were logarithmically transformed prior to analysis in keeping with the assumptions underlying the use of Pearson's correlation (ln Δ SWT%). Several measures were found

to be associated with $\ln\Delta\text{SWT}\%$, including: baseline SWT performance, subject weight, subject sex, use of β -blocker therapy, presence of diabetes, $\ln\text{HF}$, LF:HF, SDNN and RR interval. When these measures were entered into a stepwise regression analysis HF(ln) was found to be the only independent predictor of $\ln\Delta\text{SWT}\%$ (See Equation 10-1 and Table 10-5).

$$\ln\Delta\text{SWT}\% = 0.414\ln\text{HF} + 1.498 \quad \text{Equation 10-1}$$

Table 10-5 Summary of stepwise multiple regression analysis for $\ln\Delta\text{SWT}\%$.

	$\ln\Delta\text{SWT}\%$		
	<i>B</i>	<i>R</i> ²	<i>P</i>
HF(ln)	0.463	0.215	0.017

To ascertain the robustness of the predictive capacity of HFln, the regression analysis was repeated, this time using the logarithm of change in shuttle test performance in metres ($\ln\Delta\text{SWTm}$). Repeating the process described above resulted in the identification of three significant predictor measures (See equation 10-2 and table 10-6)

$$\ln\Delta\text{SWTm} = 0.874\text{Sex} + 0.372\ln\text{HF} + 0.0137\text{Mass} \quad \text{Equation 10-2}$$

Table 10-6 Summary of stepwise multiple regression analysis for $\ln\Delta\text{SWTm}$.

	$\ln\Delta\text{SWTm}$		
	<i>β</i>	<i>R</i> ²	<i>P</i>
Sex	0.530	0.224	0.015
$\ln\text{HF}$	0.438	0.453	0.005
Weight	-0.368	0.585	0.001

10.3.4. *Low to high frequency power ratio as predictor of change in heart rate variability.*

Pearson's product moment correlation between baseline LF:HF ratio and the change in the measured over the course of rehabilitation revealed that these measures were unrelated in the CR group ($r = 0.009$, $P = 0.985$)

10.3.5. *Paired group analysis.*

For reasons of comparability, the secondary analysis of pre- and post-test HRV data was carried out using the statistical analyses more commonly reported in the literature (paired *t*-test, CR and CT groups). First, no changes between any pre- and post-test HRV measures were evident in the CT group ($P > 0.05$). In comparison, analysis of the CR group data, paired *t*-tests revealed significantly ($P < 0.05$) increased: LF(ln) and HF(ln) in the frequency domain and SDNN and rMSSD in the time domain. Only the LF:HF ratio remained unchanged from test to retest.

10.3.6. *Pre- and post-test analysis by patient subgroup.*

The final analysis was a subgroup analysis between groups based on reason for attending CR. The first group split was into patients who had/had not had an MI, regardless of any surgical intervention, elective or otherwise. Independent *t*-tests revealed that the MI group ($n = 18$) and the non-MI group ($n = 20$) did not differ on any HRV measure at baseline nor was there any difference in the changes of any HRV measure (Δ HRV) between groups from pre- to post-test.

Dividing the patients into groups according to whether any revascularising surgery had been received, regardless of the occurrence of a previous MI showed that LF:HF was significantly higher at baseline in the patients who had undergone a surgical procedure

compared with the those who had not (LF:HF = 0.274 vs 0.981, $t = 3.09$, $P = 0.004$). This difference remained statistically significant when controlling for the presence or absence or previous MI ($P = 0.014$). There were, however, no differences in Δ LF:HF between these groups regardless of whether, previous MI, and/or baseline LF:HF were controlled for.

10.4. Discussion.

The findings of this chapter will be subdivided into discreet sections and addressed with reference to previous data separately. Within each section, the unique nature of each finding and the methodology used to elicit it will be briefly reviewed and justified. Lastly, each section will contain a brief conclusion and recommendations for future possible empirical research related to the findings presented.

10.4.1. Changes in routinely assessed risk factors due to cardiac rehabilitation.

Analysing the effectiveness of the CR was not the primary aim of this study. However, to ensure comparability with previous data, it is necessary to ascertain whether the CR programme has had similar effects on its patients to those expected and previously reported. Of those most commonly reported, which were recorded in the present study four were chosen for analysis here: exercise capacity, percentage improvement and improvement in metres walked on the 10-m shuttle walking test, heart rate recovery, and the psychological measures of anxiety and depression. These factors were only measured in the CR group and analysis of differences between pre- and post-rehabilitation measures showed significant improvements in all scores. This demonstrates change in these measures over the course of the present CR programme. However, as these measures were not made in the control group the degree to which the CR programme is responsible for these changes cannot be assessed. It is difficult therefore to provide a quantitative assessment of the effectiveness of the CR programme

in reducing these risk factors and fulfilling the criteria for outcomes set nationally (Jolliffe *et al.* 2000).

10.4.2. *Changes in resting heart rate variability.*

The primary aim of the present study was to determine whether cardiac rehabilitation was able to significantly, positively modify autonomic function measured by short-term HRV. The present data show significant changes in numerous HRV measures made at rest and for simplicity, these will be addressed separately.

10.4.2.1. Changes in spectral measures.

High frequency spectral power (HF) is modulated almost uniquely by the action of the vagus on the SA node (Taskforce 1996). Increased HF, demonstrating increased vagal modulation of heart rate, is commonly observed in conjunction with bradycardia following exercise training (chapter eight). It has previously been suggested that this may be a beneficial response to exercise training (Davy *et al.* 1996; Davy *et al.* 1997) and has even been described as ‘a non-pharmacological, antiarrhythmic intervention’ (Billman 2002). Animal data support this description by showing a significantly reduced occurrence of fibrillation in exercise trained dogs with higher vagal modulation evidenced by higher HF (Billman *et al.* 1984).

In the present study HF(ln) increased by about 10% in the CR group. Although this change was not statistically significant when compared to changes in the CT group, this was due mainly to a wide distribution of scores in the latter. When analysed by the more commonly used method of paired *t*-test, HF(ln) showed a significant increase over the course of rehabilitation programme in the CR group. Previous studies using 24-hour ambulatory recordings have failed to show any such change in HF (Leitch *et al.* 1997; Stahle *et al.* 1999; Tygesen *et al.* 2001). However, the lack of control afforded by

this method of HRV analysis may be a confounding factor. Using short-term data collection techniques and controlled conditions as simple as lying supine in a quiet room, will give a better indication of HF power. Under such resting conditions HF will be optimised and may be easier to study.

It can be argued that taking one 5-min measure of RR intervals under standardised, controlled condition gives a more accurate picture of autonomic control in that condition than the average of 480 separate 5-min epochs recorded under many different physiological conditions. Oddly, no studies which have used short-term analysis of HRV have attempted to demonstrate an increase in HF following CR and have instead relied on the use of normalised units. (La Rovere *et al.* 1992; Duru *et al.* 2000; Malfatto *et al.* 2001; Lucini *et al.* 2002). This is a phenomenon worth mentioning as HF power (expressed as both logarithmic (ln) and raw (ms^2) units) is known to be a predictor of future cardiac risk in this population, whereas HFnu has not been assessed. The information provided by HFnu also duplicates to some degree that given by reporting the LF:HF ratio, which is common in all of the above studies. With no quantitative pre- and post-cardiac rehabilitation data to compare with the present values, the present study is unique in this finding using short-term HRV data collection. The effects of rehabilitation on HF must, therefore, be assessed in terms of impact on future risk. Low HF power is a known risk factor when derived from 24-hour ambulatory measures (Bigger *et al.* 1992; Bigger *et al.* 1993; Lanza *et al.* 1998) and short-term measures of HF (Bigger *et al.* 1993). Positive predictive accuracy for all cause mortality and sudden cardiac death similar to other HRV measures such as VLF, LF, and LF:HF has been demonstrated, although data are limited (Bigger *et al.* 1993; Faber *et al.* 1996). Bigger *et al.* (1993) found that post-MI patients with $\text{HF} < 20 \text{ ms}^2$, derived from a 5-min resting ECG, were 1.9 times more likely to die (all causes) than those above this cut point. This risk was slightly higher for cardiac death (risk ratio = 2.3). Unfortunately, operator-receiver characteristics of the survival curves for HRV measures in this study were not calculated and values were instead, based upon previous research. This and the fact that

values were dichotomised and not continuous makes interpreting the findings of the present study difficult. It seems justifiable, however, to state that any increase in HF power due to cardiac rehabilitation is likely to decrease risk of mortality, particularly due to cardiac death. The magnitude of such an effect is difficult to establish due to the paucity of data concerning short-term HRV as a risk factor following MI. Faber *et al.* (1996) simply used short-term measures as a 'pre-screening' tool for 24-hour recordings. This provided greater sensitivity in the latter but again makes it impossible to quantify the real usefulness of short-term measures in risk stratification.

In the present study, changes in low frequency spectral power were greater in the CR group compared with the CT group. These values also increased in the CR group when compared with their own baseline measures. Again, LF in raw units has not been specifically investigated previously by authors using short-term recording methods. This is probably due to the lack of knowledge concerning the exact physiological meaning of LF oscillations at rest, although the reflection of Meyer waves (oscillations in blood pressure) has been put forward. However, LF is more commonly expressed in the literature in normalised units (nu). Due to its rise in response to orthostatic and pharmacological sympathetic stressors, it is a well established measure of sympathetic activation (Pagani *et al.* 1986). Due to the association of LFnu with sympathetic modulation of HR, a reduction in LF has become a proposed therapeutic target and a commonly hypothesised, beneficial effect of exercise training in post-MI patients (La Rovere *et al.* 1992; Malfatto *et al.* 1998). Both these authors demonstrated significant decreased in LFnu during CR. Malfatto *et al.* also showed an additive effect of β -blocker therapy and exercise training on reducing LFnu. Again, in the present study LFnu was not assessed as it, and its inversely related measure HFnu, are more easily represented and interpreted when given as the LF:HF ratio. This value is usually provided as a measure of sympathovagal interaction and balance, although the semantics and physiological underpinnings of any such phenomena as sympathovagal balance are debatable (Goldberger 1999).

Despite the lack of research into LF power using short-term HRV recordings, it is also a known risk factor for future cardiac event in a number of pathologies such as heart failure (La Rovere *et al.* 2003) and again, following MI (Bigger *et al.* 1993). The data in these risk stratification studies suffer the same shortcomings described in terms of HF power. Only one study has provided evidence that low LF ($< 35 \text{ ms}^2$) derived from short-term recordings is associated with increased risks of all cause mortality with (RR = 2.8) and cardiac death (RR = 3.0). Again, therefore, only a general statement that increased LF power in raw units (ms^2) is a likely beneficial effect of rehabilitation in reducing risk can be made. It can also be stated however, that the risk ratios for LF are greater than those for HF. One ambulatory study has shown no significant differences in HF power between surviving and non-surviving post-MI patients despite large statistically significant differences in LF (Quintana *et al.* 1997). On this basis, it seems that increasing LF may be a more beneficial therapeutic target for CR.

Previous data regarding the ability of CR to alter LF show no significant effects of therapeutic exercise on this measure. Using 24-hour ambulatory recordings, Mazzuero *et al.* (1992) found no discernable change in LF (ms^2) from test to retest. Pardo *et al.* (2000) found a mean change of 0.4 log units for LF over the course of CR, but this failed to reach statistical significance. Neither of these studies utilised a control group. Leicht *et al.* (1997) compared $\Delta\text{LF}(\ln)$ in controls and CR patients but found no difference. This was despite a mean $0.32 (\pm 0.18)$ log unit increase in the CR group. The lack of statistical significance may have been due to the relatively large ($0.17 \pm 0.13 \ln$) increase observed in the controls. In the present study the value of $\Delta\text{LF}(\ln)$ in the CR group was 0.59 log units, larger than both previous values and the change in the CT group was very small and actually negative (-0.14 , C.I. $-0.577 - 0.269$). Statistically, it should be noted that the value given here was corrected by ANCOVA to account for the influence of baseline LF(\ln) values. Therefore, a combination of the larger subject numbers, due to *a priori* power calculations and the method of statistical analysis used,

may be responsible for this unique finding. Other factors related to the intervention and the HRV sampling methodology may also be at work here and these will be highlighted in the more general discussion of the study findings.

The LF:HF ratio, a measure of sympathovagal balance, has repeatedly been shown to be a predictor of adverse outcome in post-MI patients (Bigger *et al.* 1993; Lanza *et al.* 1998; Huikuri *et al.* 2000). However, somewhat counterintuitively, a high LF:HF ratio, which is indicative of predominant sympathetic modulation of the SA node has not been found to be a risk factor. Instead, increased risk of adverse event is associated with a lower LF:HF ratio. Optimal cut points of 1.6 (Bigger *et al.* 1993; Huikuri *et al.* 2000) and 0.95 (Bigger *et al.* 1993; Lanza *et al.* 1998) from 24-hour recordings have both demonstrated significant association with numerous adverse clinical endpoints. The fact that LF:HF ratio remained unchanged in the present study can therefore be seen as a positive outcome of CR.

A point worthy of mention here is that in the data concerning exercise intervention in healthy subjects, there is a common conception that decreasing LF:HF (tipping the balance of SA node modulation toward vagal predominance) is a beneficial outcome. Cross-sectional comparisons show a lower LF:HF ratio in athletes compared with controls, (Bonaduce *et al.* 1998) described previously as a 'parasympathetic cardioprotective balance' (Gallagher *et al.* 1992). Additionally, longitudinal data have shown that exercise can decrease LF:HF ratio (Portier *et al.* 2001).

This paradox warrants a brief explanation. It seems that in the general population, where there is significant power in both LF and HF bands ($>100 \text{ ms}^2$), that a balance between the two is indicative of healthy autonomic control and therefore, desirable. In chronic sympathetic predominance at the SA node the overall oscillation in heart period is greatly reduced. This has been demonstrated experimentally in heart failure patients (van de Borne *et al.* 1997; Notarius *et al.* 1999). The predominant sympathetic

modulation heart period leads to reduced oscillations of nervous genesis, (in all frequency bands) but there is a slight preservation of HF oscillations due to other inputs such as mechanical stimuli. These are similar in nature to those observed in the denervated transplant hearts (Ordway *et al.* 1982; Smith *et al.* 1989; Bernardi *et al.* 1990).

This preservation of HF power, simultaneous with reductions in total and especially LF power produces a low LF:HF ratio. This is very similar to that reported in high intensity, rhythmic exercise (Perini *et al.* 1990; Casadei *et al.* 1995; Perini *et al.* 2000) and represents a parabolic relationship between level of sympathetic modulation and the LF:HF ratio. Such a relationship makes interpretation of this ratio and indeed normalised values for LF and HF very difficult in clinical populations with sympathetic overactivity problematic.

Cardiac rehabilitation did not significantly increase subjects' LF:HF in the present study; the ratio instead, tended to decrease in the CR group and remain unchanged in controls. This can be explained by both the timing of the tests and to a degree, the conditions under which they were made. At rest, in the supine condition, vagal modulation of the SA node is the predominant influence on heart period. Therefore, any increase in vagal modulation, which manifests itself as HF oscillations will become obviated in this type of test compared with the mean HF power of all the 5-min periods in a 24-hour, ambulatory electrocardiogram. A further justification to support the beneficial role for decreased LF:HF ratio in this population is the test timing. The long duration from MI and/or surgery to entry into CR means that the acute phase of sympathetic ANS activation, which is well documented following MI (Bigger *et al.* 1991; Bigger *et al.* 1992; Bigger *et al.* 1992; Bigger *et al.* 1995) would have occurred weeks and in some cases months prior to the test. This, coupled with the high percentage of patients receiving β -blockade therapy which decreases sympathetic ANS activity and increases global and vagal HRV measures (Malfatto *et al.* 1998) means that

they are a somewhat different population from those studied previously. Any meaningful, comparison of LF:HF ratios in acute MI patients, MI patients months after their event and revascularised CAD patients is impossible. However, mean values for LF and indeed HF in the present study were higher than those gained from 5-min recordings previously (Bigger *et al.* 1993), indicating that the reversal of sympathetic activation which occurs spontaneously after MI may have already occurred in the present population. This fact and the lack of any change in the CT group means that any alterations in autonomic profile evidenced here by increased HRV are due to CR. The findings of this study are therefore, unique in this respect.

It must be taken into account, however, that in the present study the subject group was a mixture of post-MI patients and patients who have had elective surgery (CABG, PTCA). The majority of previous studies have dealt exclusively with post-MI patients. Indeed many of the patients in the present study with previous MI had also received some form of surgical intervention. It may be that CR offers more beneficial effects on the autonomic profile of patients who have undergone elective cardiac surgery than post-MI patients. The results of this *post hoc*, subgroup analysis will be discussed later.

10.4.2.2. Changes in time domain indices of HRV.

The standard deviation of normal-to-normal intervals (SDNN) is the most widely investigated measure of HRV in post-MI patients, due probably to the simplicity of its calculation and the fact that valid measures can be obtained from both long and short-term recordings (Taskforce 1996).

A number of previous studies have shown increases in 24-hour SDNN due to CR (Mazzuero *et al.* 1992; Tygesen *et al.* 2001; Lucini *et al.* 2002) although the data are not entirely homogenous (Pardo *et al.* 2000; Lucini *et al.* 2002). Leitch *et al.* (1997) found a large (36 ± 6 ms) increase in SDNN from test to retest in their CR group. This change

was however, not significantly different from that of their control group (40 ± 9 ms). This is a clear illustration of the spontaneous recovery of HRV following MI first demonstrated by Bigger *et al.* (1991). Patients in the study of Leitch *et al.* were all investigated on days 5 – 7 following MI. The values obtained in this study can unfortunately not be compared with those of the present study due to the use of 24-hour Holter monitoring in the former. What the study of Leitch *et al.* clearly demonstrates, is the importance of using some sort of control group in such a study, particularly if patients are to be studied in the acute phase after MI.

Using short-term data collection, Duru *et al.* (2000) found no statistically significant increase in SDNN during the training period. This was despite mean values increasing from 30 ± 25 at baseline to 36 ± 21 at one month and 45 ± 38 at two months. This represents a 50% mean increase but the SD values show a degree of heterogeneity in this response. Unfortunately, the parametric analysis used in this study (2 x 3 repeated measures ANOVA) may have been inappropriate due to these homogeneous variances and a type two error may have occurred. Notwithstanding this, the authors provide no details about which *post hoc* statistical comparisons were used, making it difficult to discern why such a large mean difference did not result in a statistically significant result. What can be discerned from these data however is an effect size of $d = 0.48$ (Cohen 1988) which represents a moderate effect of rehabilitation on SDNN.

In the present study, the CR group showed significantly greater improvements in SDNN than the CT group. Using a paired analysis, there was also a significant increase in SDNN in the CR group from baseline to post-CR. These data therefore agree with the majority of previous studies which have also reported either significant or moderate effect size changes in SDNN calculated from either short-term or ambulatory ECG measurements.

Perhaps more importantly, SDNN is also the most investigated HRV measure in terms of post-MI risk. Ambulatory data have repeatedly confirmed that SDNN is a significant risk factor for all cause mortality, (sudden) cardiac death and reinfarction in post-MI patients (Bigger *et al.* 1993; Faber *et al.* 1996; Fei *et al.* 1996; Reinhardt *et al.* 1996; 1996; Zuanetti *et al.* 1996; La Rovere *et al.* 1998; Lanza *et al.* 1998; Poulsen *et al.* 2001; Liu *et al.* 2003). Data in disagreement with this supposition are rare (Huikuri *et al.* 2000; Manfrini *et al.* 2003) and in one case at least (Manfrini *et al.* 2003) are almost certainly due to the very small number of events recorded in the population studied.

Of interest within this large cumulative data set are three studies which have used both 24-hour and short-term measures of SDNN in risk analyses (Faber *et al.* 1996; Fei *et al.* 1996; Reinhardt *et al.* 1996).

Faber *et al.* (1996) used 5-min SDNN to pre-select patients for risk analysis by 24-hour HRV index (HRVi), a geometric ratio derived from the dimensions of a histogram of all RR intervals in an ambulatory tachogram. These authors found that prediction of all cause mortality, sudden cardiac death and arrhythmic death could be improved by systematically selecting patients with lower values for 5-min SDNN. These authors did not calculate relative risk scores for 5-min SDNN. However, they did calculate differences between patients with or without the above adverse events were larger for 5-min recordings than for the entire 24-hour data strip. For ease of interpretation here, these differences have been transformed into effect sizes (d) (Cohen 1988).

For two year overall mortality the effect size for 5-min SDNN was $d = 0.91$ whereas for 24-hour HRVi it was $d = 0.86$. Differences between subjects suffering/not suffering cardiac death over the same period were almost identical between 5-min SDNN ($d = 0.69$) and 24-hour HRVi ($d = 0.70$) as was the case in predicting 2-year arrhythmic death (5-min SDNN, $d = 0.81$ vs 24 – hour HRV, $d = 0.82$). These data show that SDNN derived from a simple 5-min recording is as sensitive a diagnostic tool as HRVi

derived from a 24-hour tachograms. These authors suggested that short-term measurement of SDNN may be a powerful screening tool when used to reduce the number of 'expensive and cumbersome' HRV measurements made.

Reinhardt *et al.* (1996) directly compared the standard deviations of RR intervals recorded over a 5-min period with those recorded over a 24-hour. When comparing these values in patients with or without arrhythmic events over the follow up period, 24-hour SDNN (and as reported by Faber *et al.*, HRVi) did not differ between groups (effect size, $d = 0.07$). However, when the SD of intervals from a 5-min stationary ECG recording was analysed the effect size was larger ($d = 0.63$) and statistically significant ($P = 0.02$). Unfortunately, neither of these measures was a significant independent predictor of arrhythmic event in the multivariate analysis performed as another time domain variable (RMSSD) was a powerful predictor (see discussion of this measure below). Fei *et al.* (1996) quantified the relationship between 24-hour HRVi and 5-min SDNN and found only a moderate correlation coefficient ($r = 0.51$). In risk analysis, 5-min SDNN was found to be a significant prognosticator of all cause mortality in 700 post-MI patients. However, sensitivity and specificity of 24-hour HRVi were greater than 5-min SDNN. The authors concluded again that short-term recordings of SDNN could not replace 24-hour recordings but that they may be used as a pre-screening tool to improve cost effectiveness.

One major problem with two studies (Faber *et al.* 1996; Fei *et al.* 1996), which coincidentally use the same retrospective data set of 24-hour tapes, is the data collection method for short-term SDNN. For example, in the present study, and throughout the scientific literature, short-term data recordings are made at rest, under controlled laboratory conditions. This ensures the stationarity of the ECG signal and reduces the impact of external influences such as mental and physical stress, movement, breathing rate and even temperature. In both the above studies, the 5-min segments were extracted from the same 24-hour tachogram with which they were later compared.

Notwithstanding the statistical problems associated with comparing data that are not derived under conditions of mutual exclusivity, this data collection method does not conform with the recommendations put forward by the 1996 Taskforce for collection of short-term HRV data. This is not mentioned in the studies of Fei *et al.* or Faber *et al.* despite the fact that two authors, common to both papers are lead members of the aforementioned Taskforce. No study to date has compared the prognostic power of short-term HRV measures, recorded under controlled conditions with 24-hour ambulatory data.

Despite differences in the prognostic power of 24-hour and short-term SDNN, the latter is still a significant predictor of risk in post-MI patients. The optimal cut-point produced in the study by Fei *et al.* was < 23 ms. Of interest here is that at baseline, this value represented the median (50th) percentile of SDNN values in the CR group. Post-CR, the median value had increased to 27 ms and 23 ms was now representative of the 40th percentile in the distribution. This change represents a positive influence of CR on the risk of a future cardiac event in the present population.

As mentioned previously, the vagally mediated time domain measure of RMSSD is a significant predictor of risk in post-MI patients (Reinhardt *et al.* 1996). An optimal cut-off point of < 36 ms was found for 24-hour RMSSD and in a separate analysis it was demonstrated that an increment of 10 ms in RMSSD produced a $> 5\%$ decrease in risk of adverse cardiac event in the 6-months following MI. These data are from 24-hour ambulatory measurement; few others are available for comparison and none are from short-term measures. Mean values of RMSSD in the present study increased from 20 to 27 ms due to CR. Values for RMSSD are smaller when derived from short-term measures and therefore an accurate assessment of the impact on future risk is not possible except to say that it is probably larger than the same magnitude increase in 24-hour measures. As 7 ms would have decreased the risk by approximately 4% according

to the previous values (Reinhardt *et al.* 1996), it can be assumed that the effect on future risk here is probably >5%.

10.5. Conclusions.

Previous studies using both 24-hour and short-term HRV recording methodologies have shown mixed effects of CR on HRV measures. The majority of studies report favourable changes in at least some of the many measures made but in all cases there are problems with these data. A common problem is the failure to employ any form of control group. A second problem lies in the forms of statistical analyses undertaken; using paired analysis of pre- vs. post-test scores alone does not account for any changes in HRV measures that may occur spontaneously. Where analysis of differences in magnitude of change between a rehabilitation and a control group have been used, failure to control for the possible confounding effects of baseline HRV measures may previously have resulted in failure to detect change.

This study is, therefore, unique in a number of respects. First, the present data were gathered on patients entering rehabilitation a typically longer time after MI and/or surgery than those patients studied previously. This is likely to have reduced the effects that any spontaneous return to recovery after MI or surgery may have had on the present results.

Second, a control group of outpatients was used, and although not identical in disease aetiology to the CR group, they were similar in age, underlying cause of disease, (ischaemic) and use of pharmacotherapy. For the reason above (time to entry to cardiac rehabilitation) it can be argued that it was not necessary to use closely matched control population.

Third, it appears that this is the only study concerning the effects of CR on HRV in which power analysis has been used to calculate the necessary sample sizes to detect a significant difference. Although this calculation was at best, coarse, it was based on the mean effect sizes of a number of previous studies and therefore reasonably robust. Notwithstanding the accuracy of this calculation, the presence of any estimation of sample size is a methodological advance on all previous data.

Fourthly, the statistical analysis undertaken in this study is rare in the existing literature (Leitch *et al.* 1997). By using a control group and examining differences in Δ HRV between CR and CT groups it was possible to control for differences in baseline HRV between and within groups. This analysis revealed that for certain measures, baseline values are significant covariates which should be controlled for in such comparisons. It is of note, that for purposes of comparability with previous data, a paired comparison was also made. This showed significant (or very near to significant) differences in all the HRV measures that were expected to be altered by rehabilitation. The significance of these results is probably due to the rigorous planning of this investigation.

Therefore, using precise, rigorous methodology, it was possible to demonstrate that an eight week cardiac rehabilitation programme, significantly altered all standard short-term HRV measures with the exception of the LF:HF ratio. These differences were significantly greater than those of the control group, who undertook no rehabilitative exercise. The 95% confidence intervals for the changes in these HRV measures were all positive indicating that response to rehabilitation was homogeneous.

Many positive psychological and physiological changes are known to occur in response to cardiac rehabilitation programmes such as reduced anxiety, increased exercise capacity, reduced heart rate, and blood pressure. All of these measures have also been identified as risk factors for future cardiac events. Low levels of many of the HRV measures recorded in the present study have also been identified as significant

independent risk factors for future adverse cardiac events. The present data demonstrate the ability of cardiac rehabilitation to increase all these identified measures, showing a further beneficial effect of rehabilitation in reducing further risk.

10.5.1. *Limitations.*

The major limitation of the present study is the nature of the control group employed. Ideally a control group should be as identical as possible to the experimental group to which they are being compared. This matching should include physiological characteristics and in clinical populations, disease aetiology and previous treatments and interventions. Due to ethical constraints, it is not possible to randomise patients to CR and CT groups as defined here. Therefore, we feel that by using outpatients with ischaemic complaints, a common underlying aetiology to that which results in MI and the need for CABG and PTCA surgical interventions, a fair comparison has been made. This is especially true due to the long time course from surgery and/or MI to onset of rehabilitation.

A second limitation is the heterogeneity of the CR group themselves. Due in part to the geographical proximity of the CR centre to a national centre for cardiovascular treatment, a larger proportion of CR patients in the present study had undergone surgical interventions than reported in previous studies. Due to small subgroup numbers and the *post hoc* nature of any analysis, no meaningful assessment of the impact of antecedent subject characteristics in terms of disease statement and treatments was possible here. This is true at least if statistics comparable to those used in the main body of the study are to be used. However, appendix five contains results of a *post hoc* comparison made using visual analysis of means and 95% C.I.s. It should be noted that these data are preliminary only, they were not a planned comparison in this study, no power analysis was performed and therefore, are meant as only a demonstration of any potential differences with the possibility of some future work examining any effects shown.

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CHAPTER 11. SUMMARY.

The body of this thesis is a series of nine chapters which consists of systematic reviews, meta-analytical and empirical work undertaken over two and a half years. These chapters are deliberately diverse in their methods of data collection, data treatment, analysis and most of all, in terms of the populations they represent. There is, however, a clear underlying theme running through these works concerning levels and changes in measures of heart rate variability.

From a philosophical viewpoint the use of such generalised nomenclature as heart rate variability to describe the methods employed in this thesis is wholly inadequate. The reader will realise that the multitude of measurements which can be derived from a single, short recording of heart period intervals make discussion of heart rate variability conceptually impossible. To avoid discussing each of the possible measurements (of which there are over twenty) which have been mentioned in this thesis, it is necessary to generalise to some degree. To do this, the HRV measures which have been used will be classified in two ways.

11.1. Absolute measures of heart rate variability.

The first categorisation is absolute measures, examples of which are SDNN (ms) in the time domain and LF or HF (ms^2) in the frequency domain. When studying these measures and their behaviours, a simple, 'more is better' viewpoint can be assumed. Low levels of these values are risk factors for numerous adverse outcomes in various clinical populations; there is no evidence to suggest increases in these values are associated with any risk or adverse event.

In terms of the effects of exercise on this category of HRV measurement, patterns are clear. Chapters six and seven showed that acutely during exercise as heart rate increases, all these measures decrease almost linearly as a function of exercise intensity. Longitudinally, the literature reviewed in chapter four gave mixed results concerning the efficacy of exercise training to increase absolute measures. For this reason it was felt necessary to meta-analyse these data in chapter seven. The meta-analysis of these data (chapter seven) showed large and significant effects for cross-sectional differences for SDNN and HF between active and inactive subjects. This analysis also showed that exercise interventions can significantly increase both SDNN and HF with a consequent increase in RR interval. The findings of this study were original, clarifying the debate within the published literature.

From the observed relationship between absolute measures and RR interval, especially under conditions of pharmacological blockade, it is clear that the majority of variation in heart rate, which these measures represent, is vagally mediated. Even low frequency spectral power, originally promoted as a sympathetic indicator, is clearly strongly influenced by the vagus. Given this relationship, it seems unsurprising that low levels of vagal activity and/or increased levels of sympathetic modulation (which reduce absolute measures) are risk factors.

On this basis, it can be argued that increasing these measures is beneficial in reducing risk and that such an effect would be particularly beneficial in subjects with attenuated levels of absolute HRV measures.

Chapters nine and ten both used exercise interventions in an attempt to promote increases in absolute measures of HRV. Patients with peripheral vascular disease (chapter nine) were found to have greatly attenuated levels of absolute HRV measures. Although large between-subject variation was evident, many subjects were found to be below previously quantified, high risk values. Exercise intervention in these patients

was not able to produce a significant increase in any measure of HRV despite significant improvements in walking capacity due to supervised exercise. Comparing these findings with those from previous clinical exercise interventions, it was proposed that the intermittent nature of the exercise may have been the reason for the lack of change observed.

In chapter ten, post-MI and CAD patients entering cardiac rehabilitation were also found to have low levels of HRV. Eight weeks of cardiac rehabilitation was successful in creating large and statistically significant changes in absolute HRV measures. The findings from this study were unique in that data were recorded in subjects entering rehabilitation much longer after either MI or surgery than previously reported in the literature. The homogeneity of changes in HRV measures shown is as yet, unrivalled in the literature. Almost all selected measures in this study increased significantly, where commonly only a small percent of the often numerous HRV measures reported show a significant change.

11.2. Normalised measures and ratios in heart rate variability.

Due to large interindividual differences in total spectral power (TP) and both the low and high frequency bands when they are expressed in raw units (ms^2), it is common practice to normalise spectral power in the LF and HF bands for total power. This has led to the creation of indices of vagal or sympathetic predominance and ratios. These indices represent what has now been termed sympathovagal balance (Goldberger 1999). It should be noted, as discussed previously in the literature and throughout this thesis, that no physiological evidence for any such 'balance' between the branches of the ANS exists and this term may be an example of misleading nomenclature born more out of convenience than of any physiological meaning (Eckberg 1997).

In the now seminal paper (Pagani *et al.* 1986) it was eloquently shown that 'sympathovagal interaction' could be described with accuracy by using frequency domain analysis of HRV. In particular, increases in LF:HF ratio due to sympathetic stimulation, and decreases under conditions designed to promote vagal firing led to the conclusions that LF:HF and the expression of LF and HF in normalised units could adequately describe changes in activity levels of both cardiac ANS branches.

These values were obtained largely under resting conditions in healthy humans and animals. What should be noted from studies in which subjects exercise, and when very high levels of sympathetic ANS activity are present (such as acutely following MI or during intense exercise), is that nearly all spectral power disappears. By its very nature, HRV relies on variation in the length of cardiac cycles; where no variation is present, no spectral power exists.

In chapters two and three it was concluded that more reliable measures of HRV could be obtained when normalised units were used. However, in consequent chapters (four, six and seven) it was found that recommended normalised units (Taskforce 1996) reflected poorly the expected changes in sympathetic and vagal contributions to SA node regulation during exercise. Additionally, between-study differences in the methodologies used to calculate normalised units made meta-analysis of either LFnu or HFnu impossible.

In chapters nine and ten, raw values for LF and HF power were found to be attenuated. This led to highly heterogeneous values for LF:HF power and spectral power expressed in normalised units. The risk factors identified in the literature in patients with CAD and post-MI tend to be raw spectral power and time domain measures. Normalised units and the LF:HF ratio rarely show multivariate associations with risk in these populations. No significant changes in any HRV measure were shown in the PVD patients investigated in chapter eight. LF:HF ratio was the only measure not to show significant

change in chapter nine (cardiac rehabilitation). Interestingly, low levels of LF and HF power (in raw units) have both been identified as risk factors following MI. Both these measures increased during the intervention. However, it seems that as these measures increased by broadly similar magnitudes, LF:HF remained unaffected.

Analysis of the published data for measures of LF:HF in clinical patients produces a paradox which can be related back to data from previous chapters. Firstly, many of the studies examining exercise interventions outlined in chapter four, expected to show a decrease in LF:HF ratio especially when measured at rest. Such a lowering of this measure of sympathovagal balance has been proposed by numerous authors to show a beneficial 'tipping of the balance' between branches of the ANS towards vagal predominance. This is also proposed to bring with it myocardial electrical stability and a decreased risk of arrhythmia. On this basis, lowering of LF:HF has been a hypothesised outcome of exercise interventions in healthy subjects which has been successfully demonstrated on a number of occasions.

Somewhat paradoxically, in clinical populations it is not a high LF:HF ratio which tends to predict increased risk of arrhythmia, sudden death or indeed any cause of mortality. It is in fact a low LF:HF ratio, which according to data from the healthy populations in which this ratio was originally validated should be indicative of predominant vagal modulation of the SA node.

The problem underlying this paradox is created by generalising findings from validation studies in healthy subjects to clinical populations. In both populations, the value of the LF:HF ratio is the product of LF and HF power. However, in the former, these values often run into the hundreds or even thousands of milliseconds. In the clinical populations, recorded values are commonly in tens or even single figures. In this situation a small variation in one band can give a misrepresentative or wholly erroneous value for LF:HF. It can even be argued that below a certain level of total spectral

power, the LF:HF ratio and of course values for normalised spectral units at best, do not represent what they may do in healthy subjects or at worst have little or no physiological meaning.

11.3. Recommendations.

Despite such problems and despite the lack of significant associations between risk and LFnu, HFnu or LF:HF these values continue to be calculated in many clinical populations where global HRV is greatly attenuated. Further work should focus on the nature of the relationship between LF:HF and sympathovagal balance in subjects with varied levels of total spectral power. In addition, identification of a lower ceiling for TP, below which any such relationship fails to retain a physiological association, should be identified.

In addition to and related to this recommendation, agreed normal, expected values for short-term HRV measures would be an invaluable resource for future research. Large, population-based studies, typically use 24-hour, Holter monitoring and are based in clinical populations (Bigger *et al.* 1995). Where short-term monitoring has been used, findings for raw and indeed normalised HRV values are heterogeneous. The large interindividuality in HRV measures can be viewed as advantageous, giving the measure high discriminant power. However, this same property also means that very small variations in data collection protocols create highly heterogeneous values (Fagard 2001; Kuch *et al.* 2001). This is particularly true when raw spectral powers are measured. As these are the best predictors of risk and appear to be more sensitive to change due to intervention than normalised measures, the importance of establishing population based norms should be a high priority for those researchers working in the field of heart rate variability.

11.4. Conclusions.

The overall theme of this thesis is that heart rate variability is a measurement with great potential to identify risk in a number of populations. This measure is also dynamic, in that it may be acutely altered, particularly by exercise. Chronic adaptation to prolonged aerobic type exercise can also cause significant alterations in certain HRV measures known to be risk factors in healthy subjects and in clinical populations. Finally, although the exact physiological meanings of certain HRV measures remain ambiguous, the fact that they predict risk makes them invaluable due to their strong associations and the simplicity and non-invasive nature of their acquisition. The fact that known HRV measurements which are known risk factors can be modulated successfully via exercise intervention and other disease management strategies means heart rate variability should be viewed as an important therapeutic target.

11.5. References.

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APPENDICES.

APPENDIX I. List of standard heart rate variability abbreviations, definitions and explanations of variables.

Abbreviation	Description	What the measure represents.
	Time domain measures.	
RR interval	The time (ms) between the fiducial points of successive R waves from the original ECG trace.	The duration of each cardiac cycle including all beats.
NN interval	The time (ms) between the fiducial points of successive R waves from data which has been filtered and/or beats removed and interpolated.	The duration of each normal cardiac cycle.
SDNN	The standard deviation of all the normal-to-normal (NN) intervals.	All the cyclic components responsible for variability in the period of the recording.
SDANN	The standard deviation of the average NN intervals calculated over 5 min.	All the cyclic components responsible for variability during cycles longer than 5 min.
RMSSD	The root of the mean squared differences of successive NN intervals.	Parasympathetic activity.
NN50 (NN50 count)	The number of successive intervals which differ by more than 50 ms.	Parasympathetic activity.
pNN50	The proportion of successive intervals which differ by more than 50 ms derived by dividing NN50 by the total number of NN counts. This may also be expressed as a percentage PNN50%).	Parasympathetic activity.
SDNN	The standard deviation of all the normal-to-normal (NN) intervals.	All the cyclic components responsible for variability in the period of the recording.
	Geometric measures.	
HRV triangular index	The integral of the density distribution divided by the maximum of the density distribution.	All the cyclic components responsible for variability in the period of the recording.
TINN	The width of the base of a triangle fitted to the histogram of duration of all NN intervals.	All the cyclic components responsible for variability in the period of the recording.

Poincare Plots	A scattergram of each NN-interval of a tachogram plotted as a function of the previous NN-interval.	All the cyclic components responsible for variability in the period of the recording.
Frequency domain measures.		
Total Power	Variation in NN interval below 0.15Hz. The lower band may be altered depending on duration of recording.	All the cyclic components responsible for variability in the period of the recording.
LF	Variation in NN interval 0.04-15 Hz.	Sympathetic and parasympathetic activity.
HF	Variation in NN interval 0.15-0.4 Hz.	Parasympathetic activity.
LF (nu)	The proportion of spectral power from 0.04 – 0.4 Hz which is in the LF frequency calculated by $LF / (TP - (ULF+VLF))$	Sympathetic activity or sympathovagal balance.
HF (nu)	The proportion of spectral power from 0.04 – 0.4 Hz which is in the HF frequency calculated by $HF / (TP - (ULF+VLF))$	Parasympathetic activity or sympathovagal balance.
LF:HF ratio	The ratio of LF power to HF power.	Sympathetic activity or sympathovagal balance.
VLF	Variation in NN interval <0.04 Hz	All the cyclic components responsible for variability during cycles longer than 25 seconds. Possibly variations in thermal control or the rennin-angiotensin system
ULF	Variation in NN interval below 0.0033 Hz.	All the cyclic components responsible for variability during cycles longer than 5 min. Origin: unknown.

APPENDIX II. Expanded descriptions and definitions of HRV terminology – including those used for HRV measurement during exercise.

Time domain parameters:

Statistics derived directly from either the NN interbeat intervals or the differences between successive NN intervals.

Frequency domain parameters

A mathematically complex analysis which gives information about the amount of variance (power) in the heart's rhythm explained by periodic oscillations of the heart rate at various frequencies.

Autoregressive modelling (ARM)

A method by which the time series of the NN intervals can be decomposed into the frequency domain. This method requires no a priori decisions about the spectral bands as it identifies peaks in the power spectrum.

Fast Fourier transformation (FFT)

A method by which the time series of the NN intervals can be decomposed into the frequency domain. This method requires a priori decisions about the spectral bands as it identifies peaks in the power spectrum.

High frequency power (HF)

The amount of variance of power in the heart's rhythm explained by periodic oscillations of heart rate at a frequency of 0.15-0.4Hz.

Low frequency power (LF)

The amount of variance of power in the heart's rhythm explained by periodic oscillations of heart rate at a frequency of 0.04-0.15Hz.

Very low frequency power (VLF)

The amount of variance of power in the heart's rhythm explained by periodic oscillations of heart rate at a frequency of 0.0033-0.04Hz.

Ultra low frequency power (ULF)

The amount of variance of power in the heart's rhythm explained by periodic oscillations of heart rate at frequencies below 0.0033Hz.

Poincare plots

A graphical analysis of NN data in which each NN interval from a tachogram is plotted as a function of the previous interval.

Two-dimensional vector analysis of Poincare plots.

A statistical method by which an ellipse is fitted to the Poincare plot. This ellipse is then fitted with both a longitudinal axis (axis 2) and an axis perpendicular to this (axis 1) which defines the transverse slope. The plot is then rotated anticlockwise and clockwise in turn to give the measures SD1 and SD2.

SD1

A representation of the beat-to-beat variability in HR.

After the clockwise rotation of a Poincare plot the SD of the points is calculated on the horizontal axis (2) which passes through the data centre SD1.

SD2

The standard deviation of long-term NN intervals.

After the anticlockwise rotation of the Poincare plot the SD of the data points is calculated around the horizontal axis (1) which passes through the centre of the data SD2.

LF (nu)

The proportion of LF to HF power in the total power spectra of the LF and HF bands.

TP – VLF = LF and HF. $LF / (LF+HF) * 100$.

HF (nu)

The proportion of LF to HF power in the total power spectra of the LF and HF bands.

LF%

The spectral power in the LF band as expressed as a percentage of VLF, LF and HF.

HF%

The spectral power in the HF band as expressed as a percentage of VLF, LF and HF.

HF CCV% (coefficient of component variance)

The square root of HF power divided by mean NN interval.

GSA – General spectral analysis

A term used by some authors to describe the decomposition of NN interval data into the frequency domain using either FFT or ARM.

CGSA – Coarse graining spectral analysis

An alternate method of decomposing the NN interval data into the frequency domain which separates the harmonic from the nonharmonic (1/f) HRV component

Low or PNS indicator

Harmonic spectral power in the frequency of 0 – 0.15Hz.

High or SNS indicator

Harmonic spectral power in the frequency of 0.15 – 1.0Hz

Low:High

The ratio of LO to HI, indicative of sympathovagal balance when CGSA is used.

β

See $1/f^x$

Lyapunov Exponent

Derived from the time series. The Lyapunov exponent is given by averaging the exponential rate of divergence of short segments of delay reconstructed orbit. It is positive for chaotic behaviour, zero or negative for periodic behaviour.

$1/f^x$

A plot used to estimate the complexity of a given time series. $1/f^x$ is a regression line of the inverse relationship between amplitude (or power) and frequency, known to exist all fractal processes. x is the exponent of the line, sometimes reported in the literature as beta (β).

Df

The fractal dimension of the non-harmonic component of HRV and is derived from the values of beta. $Df = 2/b-1$ for values of $1 < b < 3$. When $b < 1$ $Df = 1$. When $0 < b < 1$ $DF = \text{infinity}$.

APPENDIX III. Example sample size calculations based on reliability coefficients in chapter two.

The Taskforce (Taskforce 1996) states that a general consensus concerning the practical use of HRV in adult medicine has been reached only in the following two areas:

1. Depressed HRV as risk predictor following MI
2. As an early sign of diabetic neuropathy.

Recently the role of HRV in prognosis of patients suffering heart failure has also been identified (Stys and Stys 1998)

III.I. Sample size calculations.

All the sample sizes below are calculated using the formula provided by Lehr (1992). Quintana *et al.* (1996) list the differences found between survivors and non-survivors, 3-years post-MI. From the list of variables provided LF and SDNN have been selected to represent frequency and time domain measures of HRV. Below is a sample size table based on the CVs reported for all three instruments in the present study. It should be noted that these results are drawn from 24-hour ambulatory data and are here only as an example.

Table III-1. Sample size calculations for LF and SDNN in post-MI patients

Measure	Instrument	CV (%)	% Difference to be detected	Sample size for $\beta = 0.8, P = 0.05$, two sided.
LF	TF5	64.7	20.2	3244
LF	CT	46.9	20.2	1742
LF	CP	59.5	20.2	2851
SDNN	TF5	6.5	17.2	36
SDNN	CT	30.0	17.2	847
SDNN	CP	25.7	17.2	621

The ability of 5-min measures of HRV to predict sudden cardiac death in a post-MI population has been carried out (Faber *et al.* 1996) but only data concerning SDNN are available in a form which allows the construction of a sample size table.

Table III-2 Sample sizes for SDNN in post-MI patients.

Measure	Instrument	CV (%)	% Difference to be detected	Sample size for $\beta=0.8$, $p =0.05$, two sided.
SDNN	TF5	6.5	16.4	41
SDNN	CT	30.0	16.4	878
SDNN	CP	25.7	16.4	644

In chronic heart failure patients a highly significant absence of LF has been found (van de Borne *et al.* 1997). The table below shows necessary sample sizes to determine differences between heart failure and healthy controls.

Table III-3 Sample sizes for LF in heart failure patients.

Measure	Instrument	CV (%)	% Difference to be detected	Sample size for $\beta=0.8$, $p =0.05$, two sided.
LF	TF5	64.7	93.7	688
LF	CT	46.9	93.7	361
LF	CP	59.5	93.7	582

The above tables are examples and are not designed for use in research design. What they are intended to do is to illustrate the profound effect that reliability has on the necessary sample size in order to achieve the desired statistical power. Of particular note is the effect a low CV (such as the TF5, SDNN) has on the number of subjects needed in a research study.

APPENDIX IV. *Meta-Analysis of effect sizes for changes in global HRV and measures of sympathovagal balance.*

IV.I. *Study selection.*

Based on a search of the PubMed and Ovid databases seven studies were identified for possible meta-analysis. The entry criteria for analysis were as follows. Subjects in each study were required either to have been diagnosed as having MI or be postoperative (CABG or PTCA) patients treated due to the presence of ischaemic heart disease. Each study was required to have measured SDNN, HF or LF:HF ratio and supplied data in a form suitable for the calculation of effect size. Studies were not required to have employed a control group as it was deemed that this criterion would eliminate too many published papers. On this basis 7 papers were entered into the meta-analysis for SDNN and 6 for LF:HF ratio.

IV.II. *Statistical analysis.*

Effect size (d) was calculated using the formula described by Cohen (1982). Pooled standard deviation was drawn from the standard deviations from the experimental group's pre and post-test values.

A fixed effects model for Hedge's g was then calculated using the effect sizes presented. A significance of $P < 0.05$ was deemed statistically significant. In addition to calculating the overall fixed effect the data were divided on the basis of the type of ECG recording carried out on which the HRV data were calculated. These data were divided into two broad categories based on previous recommendations (Taskforce, 1996), 24-hour, ambulatory data collected using Holter monitoring (24-hour) or short-term data collected while the subject is under resting conditions with a relatively stable heart rate (short-term). To observe the effect of data collection method on each measure of interest the Q statistic was calculated by dividing the Chi^2 value within each group by the Chi^2 value between groups. This gives a measure of within and between-group variance analogous to that derived from ANOVA and provides a quantitative measure of heterogeneity.

Due to the low statistical power of this test a value of $P < 0.10$ was regarded as statistically significant.

IV.III. *Meta-analysis results.*

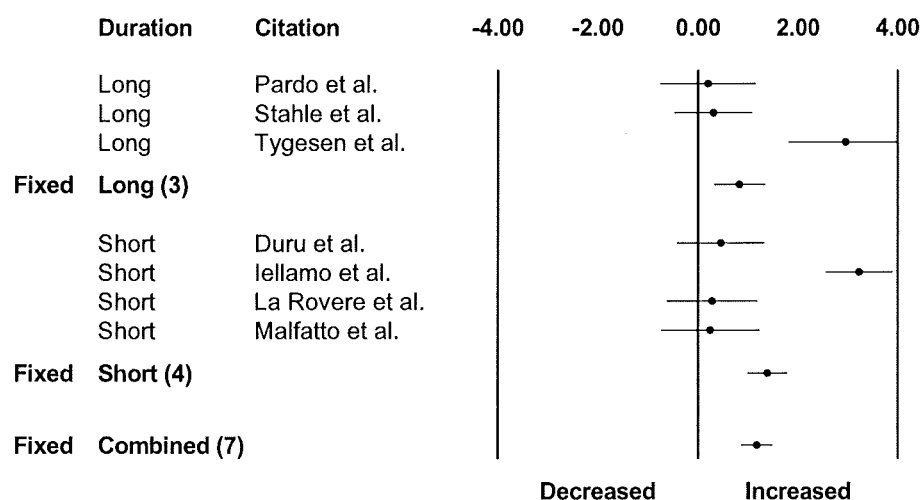


Figure IV.1 Changes in SDNN due to cardiac rehabilitation.

In total, seven trials (n=114 cases) were compared pre- and post-rehabilitation. The overall effect of rehabilitation created an adjusted Hedge's g value of 1.17, ($P < 0.001$).

Changes in SDNN remained significant regardless of whether it was derived from 24-hour ECG recordings ($g = 0.82$, $P = 0.002$) or short-term recordings ($g = 1.38$, $P < 0.00001$). The difference between these two effect sizes was found to be statistically significant ($Q = 2.96$, $P = 0.08$).

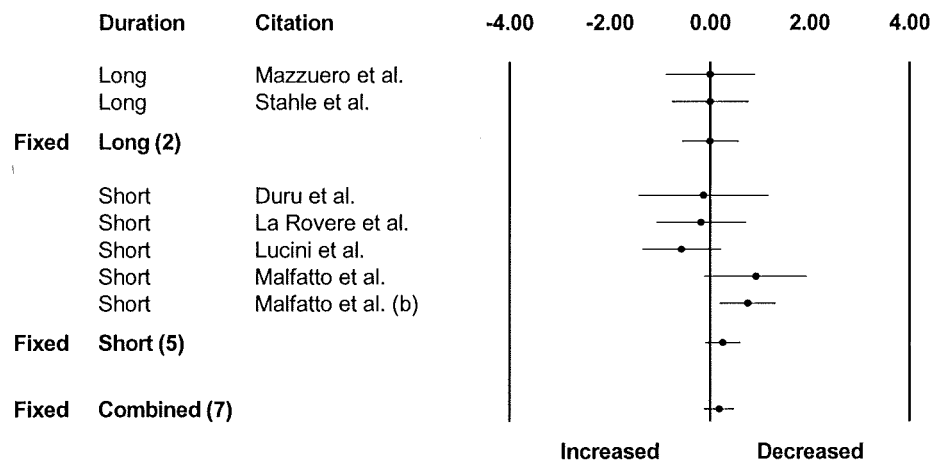


Figure IV-2 Changes in LF:HF due to CR.

Overall effect size for changes in LF:HF ratio were not statistically significant ($g = 0.18, P = 0.22$). When analysed separately, effect size for 24-hour recordings was actually $g = 0.00, P = 1.0$; short-term recordings the effect size was also nonsignificant ($g = 0.25, P = 0.15$). Calculation of the heterogeneity statistic revealed that this latter value was not significantly different to zero ($Q = 0.58, P = 0.44$) showing no significant between-group heterogeneity. However, findings from studies using short-term data collection displayed significant within group heterogeneity ($Q = 11.0, P = 0.02$)

IV.IV. Discussion.

The results of the present meta-analysis show the large variation in outcomes regarding changes in HRV measures during CR. Global measures of HRV such as SDNN are generally shown to be significantly increased. This is as expected as longer RR intervals (slower HR) that commonly accompany exercise training are often associated with greater variation. As the heart spends the majority of its time at rest under continual down-regulation from its intrinsic rate by the vagal nerve,

an increase in vagal modulation, should increase variation and therefore SDNN. This should be true for both short-term and 24-hour monitoring.

The significant heterogeneity of results between these methods may be explained by their differing sensitivities. Under short-term, resting conditions the heart rate is low, and under greatest influence from the vagus, this gives the PNS the opportunity to express its action and for this action to be manifested as increased HRV. Stress, both mental and physical accompanied by changes in behaviour and activities may be the reason for decreased sensitivity of SDNN when it is derived from 24-hour readings.

Similarly, when LF:HF ratio is derived from 24-hour recordings a mean value for each five minute period is given. The lack of any significant changes in LF:HF is therefore unsurprising as the continual modulation of the ANS between PNS and SNS dominance through daily activities is likely to obscure any change in the overall balance of the two branches. A greater effect size for shift in autonomic balance was found when short-term resting measures were used. Although not statistically significant ($P = 0.15$) it does show a trend towards an increase in LF:HF ratio in patients undergoing rehabilitation. This is however problematic as increased LF:HF is indicative sympathetic predominance at rest. Such conditions are commonly found in post-MI patients as well as those with other forms of heart disease, however, LF:HF has been reported to be higher in healthy individuals when compared with CHF and post-MI patients (Bigger *et al.* 1995). Additionally increased LF:HF is not indicative of poor prognosis in patients with heart disease (Forslund *et al.* 2002) when taken from 24-hour recordings. Where short-term assessment of LF:HF ratio is used it has been found that using a cut off point of 0.95, those patients with higher values for LF:HF (e.g. sympathetic predominance) had significantly increased mortality than those with < 0.95 values (Bigger *et al.* 1993).

It therefore seems that the goal of cardiac rehabilitation should be to decrease stable, resting measures of LF:HF to demonstrate an increased vagal predominance in patients. However, pooled data from existing studies show this value to be

increased. The underlying factors determining this could be methodological in nature and the neural and non-neural genesis of LF and HF have been discussed previously (see Chapter 3). It may be that the very small values for both spectral bands observed in this population give rise to discrepant findings. Alternatively, it is known that vagal influences contribute to the LF spectral component and the increase in LF:HF may be a consequence of this.

The reasons why LF:HF, a known risk factor for 1-year mortality should be increased by CR are not known by should be resolved. The effect is in the opposing to direction to that which would be expected, this means that sample size equations based on these findings are not possible at this time.

Based on previous data and the mini meta-analysis performed here it is possible to make the following power calculations (Table IV). The values for each part of the study are different from one another

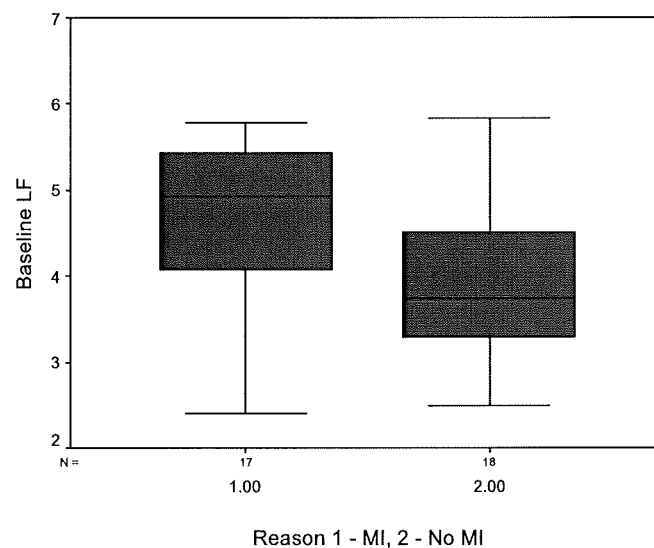
Table IV-1 Sample Sizes

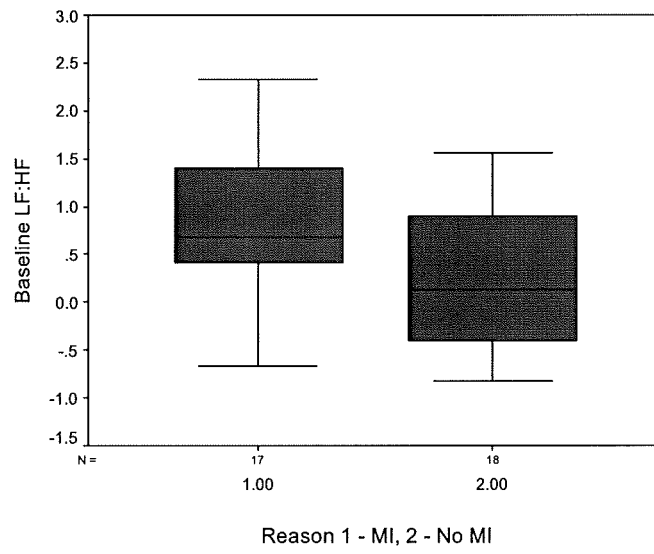
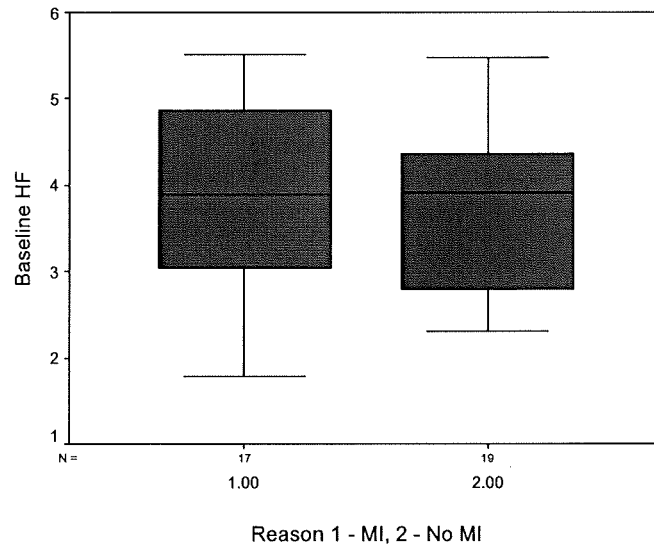
Variables to be tested.	Sample size calculation based on:	Number of subjects required at ($P < 0.05, \beta = 0.8$)
Correlation between HF power and change in aerobic performance	Based on previous data (Hautala <i>et al.</i> (2003)	(n = 22)
Correlation between LF:HF and change in autonomic function	Based on previous data (Mazzuero <i>et al.</i> 1992)	(n = 31)
Change in SDNN	Based on effect size calculations from meta analysis (7 based on Lucini <i>et al.</i> 3 based on Illemo <i>et al.</i> 44 based on Malfatto <i>et al.</i>)	(n = 18)
Changes in HF	Based on mean effect size calculations from meta analysis ² based on Lucini <i>et al.</i> 21, Mazzuero <i>et al.</i> , Pardo <i>et al.</i> .	(n = 43)
Changes in LF:HF	Based on previous data (Malfatto <i>et al.</i>).	(n = 21)

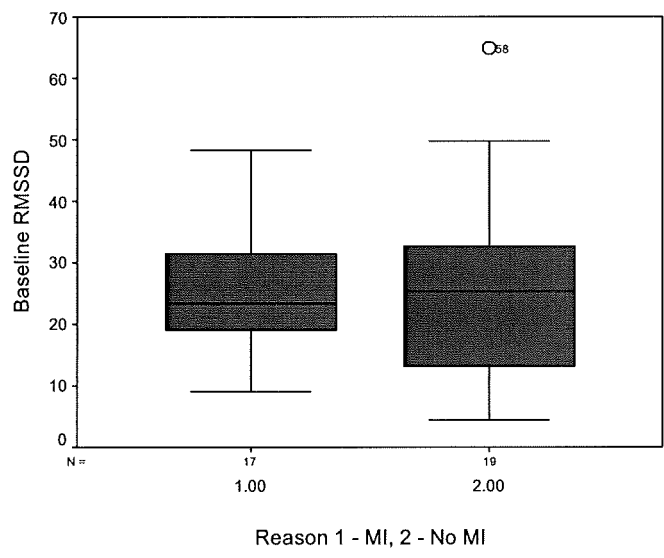
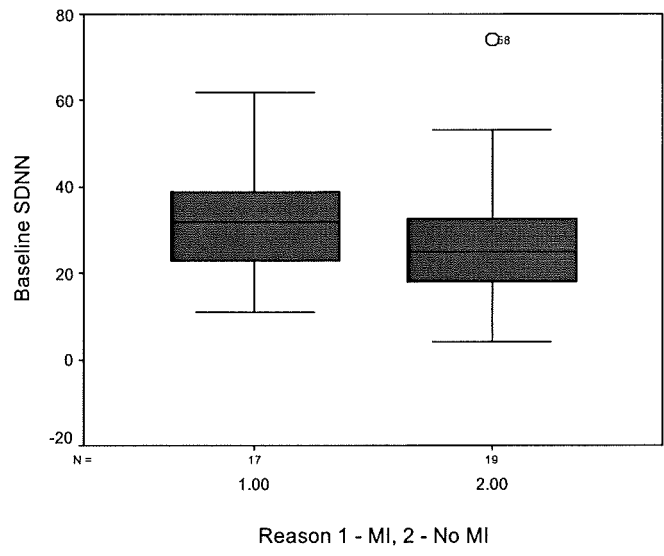
APPENDIX V. Subgroup analysis for chapter ten.

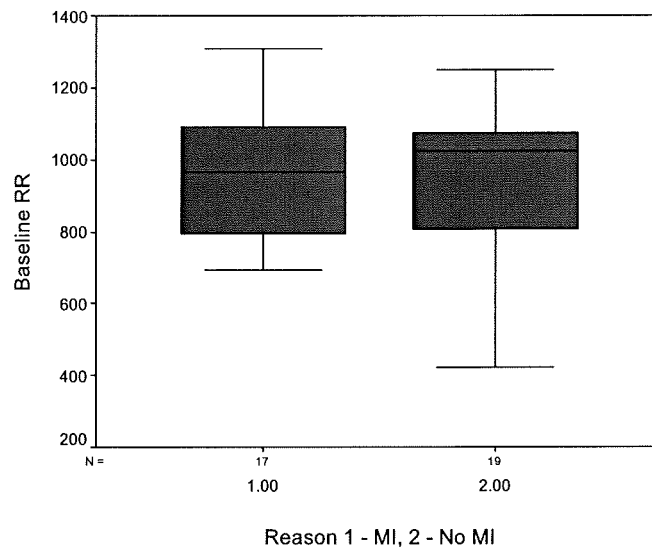
Baseline HRV characteristics of patients in subgroup 1 – those who's primary reason for attending CR was due to myocardial infarction either with or without resultant surgical procedure and subgroup 2 – those patients who's primary reason for attending CR was due to surgical procedure regardless of previous history of MI. All graphs are mean (bar), 25th, 75th percentiles (solid box), 5th and 95th percentiles (t-bars).

Box and whisker plots have been used to illustrate the following data as they provide simple presentation of mean changes and changes in dispersion from test to retest. The small patient numbers in each group negate any meaningful statistical analysis.







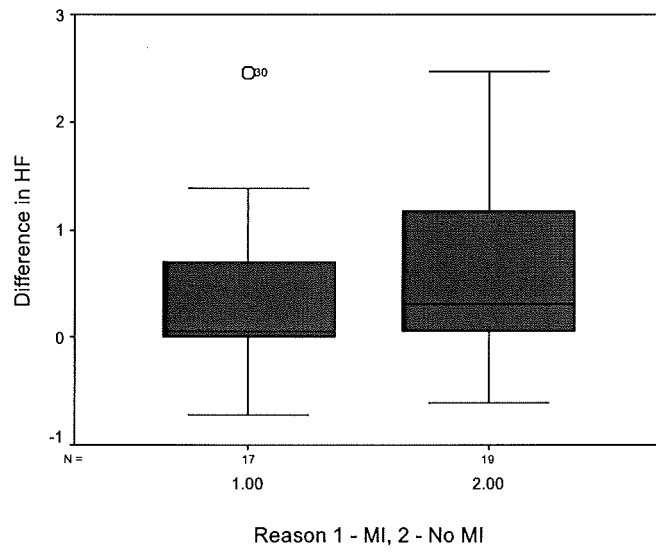
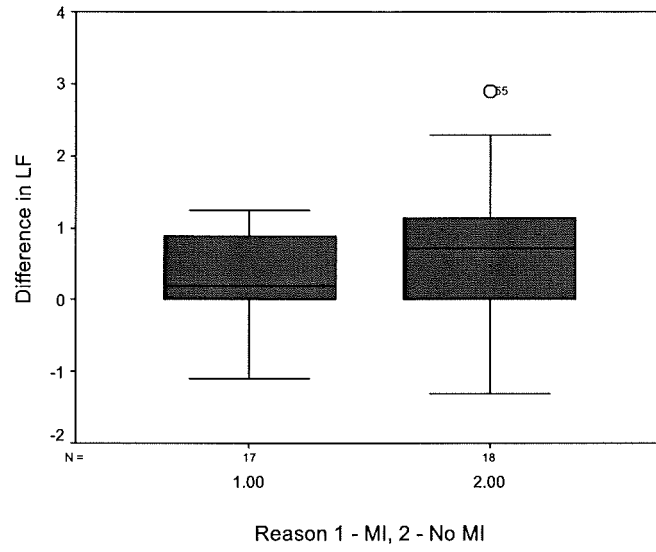


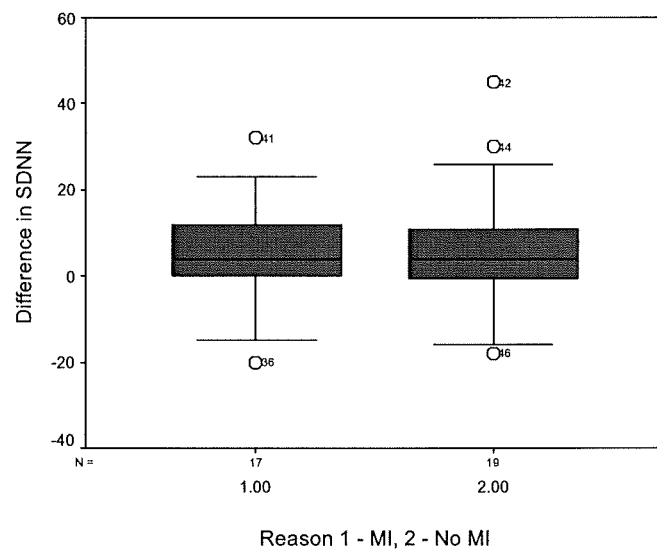
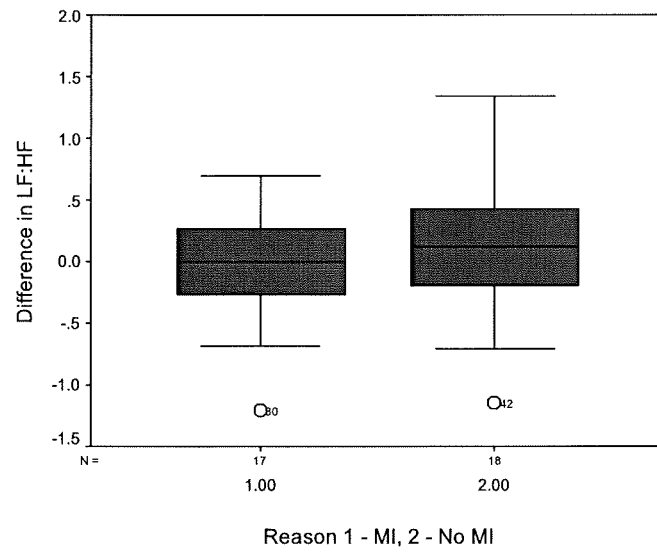
Figures V- 1 – V-7. Differences in baseline HRV values for MI and non-MI subgroups.

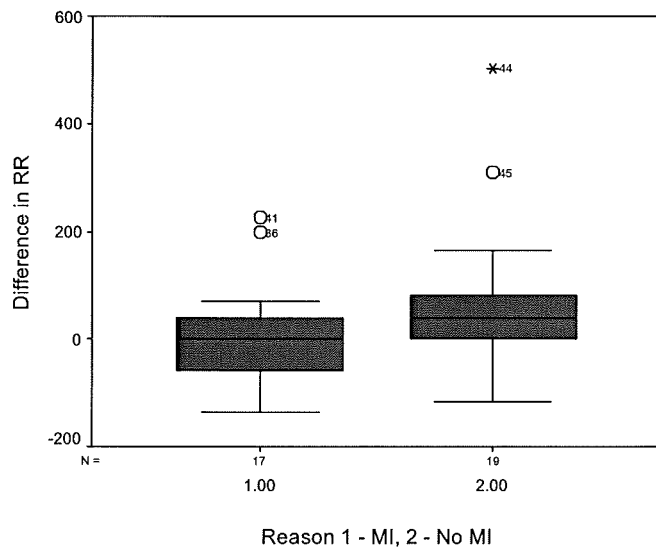
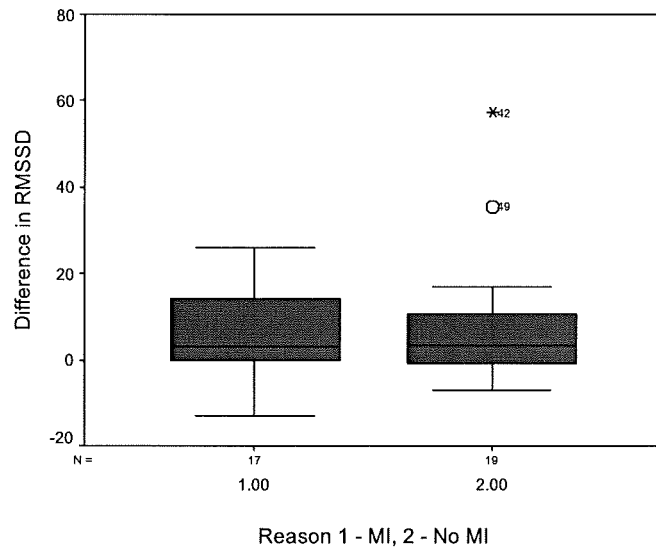
On the basis of above figures it is difficult to draw any strong conclusions concerning differences in the baseline characteristics of MI and non-MI patients. One general observation is that vagal measures such as RMSSD and HF power are lower and equal between the two groups respectively. Additionally, the LF:HF ratio which takes into account both vagal and sympathetic modulation, shows a tendency toward greater sympathetic and/or lesser vagal modulation in the MI group compared with the non-MI group.

Regardless of any differences observed, the distribution of scores for all measures in the non-MI group is uniformly larger compared with the MI group. This indicates a greater heterogeneity in autonomic control in this group. Further work is required to elucidate the possible effects the differences in baseline sympathovagal balance have on response to CR as highlighted previously (Malfatto *et al.* 2000).

All graphs are mean (bar), 25th, 75th percentiles (solid box), 5th and 95th percentiles (t-bars).







Figures V-8 – V-14. Differences in Δ HRV values for MI and non-MI subgroups.

V.I. Differences in Δ HRV between subgroups.

What can be seen from the above figures is that values for the frequency domain measures (LF and HF) increased to a greater extent in the no MI group. The LF:HF ratio which was originally lower in this group, also increased by a greater amount which resulted in a somewhat paradoxical increase in RR interval. This

increase was also of much greater magnitude in the non-MI group. It is of interest to note that, in addition to baseline values, the values for change in spectral measures of HRV were also more heterogeneous in the non-MI group compared with post-MI patients. The reasons for this pattern of change and the impact it may have on changes in HRV during CR require further investigation. Changes in time domain measures were almost identical between groups.