

# **Cortisol, Stress Responsivity and Cognitive Function in Older Adults: Relationship to Waist Hip Ratio and Metabolic Parameters**

**Nicola Jane Lasikiewicz**

Submitted in accordance with the requirements for the degree of  
Doctor of Philosophy



**The University of Leeds**

Institute of Psychological Sciences

August 2006

The candidate confirms that the work submitted is her own and that appropriate credit has been given where reference has been made to the work of others

This copy has been supplied on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement

## ACKNOWLEDGMENTS

First and foremost, I must acknowledge the excellent supervision provided by my supervisors Dr. Louise Dye and Dr. Hilde Hendrickx. I am grateful for the continued support, guidance and advice. A special thank you to Louise – thanks for your tireless commitment to this thesis and continued enthusiasm.

I must acknowledge the Medical Research Council (MRC) and Unilever Corporate Research for funding this research. I would like to thank Duncan Talbot, Jacqui, Coverly, Jonathan Powell and Lorraine Butlin at Unilever Colworth for their technical input and guidance during sample collection and biochemical assay. I thoroughly enjoyed my time in the laboratories, thank you.

Thank you to all of my volunteers (and my panellists) – without your commitment and enthusiasm for the research, this thesis would not exist.

There are a number of people I would like to thank at the Institute of Psychological Sciences, University of Leeds; Clare Lawton, Fiona Croden, Phillipa Caudwell, Alexa Hoyland, Dawn Greene and Mark Hopkins at the HARU for your support. The PG's; Tamara Turner-Moore, Therese Shepherd, Aparna Shankar, Emily Newman, Trish Holch and Rowena Handley for being good friends and for allowing me to discuss ideas and interpretations. A special thank you to Zoë, for being a wonderful friend and an emotional crutch. I will always be grateful for everything you have done for me.

A huge thank you to my Mum. You have been there for me no matter what. Thank you for your endless love and support during the last four years (and always); knowing that you were only a phone call away got me through a bad day. Dad, thanks you for being so proud of me, it encouraged me to have faith in myself and to achieve my goals. I love you both. Jean and Alan; thank you for your unconditional support and understanding. Helen and Calum; thank you for believing in me.

A very special thank you to Mark, whose unconditional love, support (and editorial assistance) has been second-to-none. Thank you for putting up with me on my bad days

and for sharing my enthusiasm and excitement on my good days. Most of all, thank you for your faith in me and for not allowing me to give up my dream, I love you.

Finally, my little stars D & C – nothing compared to the greeting you always gave me on return from a bad day at work.....

**ABSTRACT**

Basal cortisol activity is postulated to be elevated in individuals with central obesity yet this elevation is not mirrored in the basal diurnal cortisol profile. Individuals with central obesity may demonstrate an enhanced clearance capability evidenced by elevated urinary cortisol metabolites, hence basal salivary cortisol levels appear normal or reduced. Those with central obesity have been found to demonstrate an elevated cortisol response to psychological stress due to dysregulation of the HPA axis. Cognitive decrement has been observed in individuals with central obesity who show insulin resistance, high blood pressure and other features of the metabolic syndrome, however, research to date has failed to address the collective influence of elevated cortisol and metabolic risk factors on cognitive performance. A study was conducted (n=83) to explore the basal diurnal cortisol profile in those with central obesity (high waist-hip ratio) compared to those without (low waist-hip ratio). The findings demonstrated that profiles exhibited by high waist-hip ratio individuals mirrored those exhibited by low waist-hip ratio individuals, although mean cortisol was reduced. A subsequent study (n=70) explored cortisol responses to a psychosocial stressor (The Trier Social Stress Test-TSST). High waist-hip ratio individuals exhibited greater cortisol during stress induction (TSST) than low waist-hip ratio individuals. Both the stress induction procedure and actual stress responses (cortisol and blood pressure) reduced performance on the Auditory Verbal Learning Task (AVLT). Performance on a paired associates learning task was impaired in high waist-hip ratio males who demonstrated a cortisol and/or blood pressure (BP) response to the stressor/no stressor. Impairment was further evident in high waist-hip ratio males who demonstrated a cortisol and/or BP response to the stress induction. The findings presented in this thesis suggest that individuals with central obesity exhibit altered basal and stress induced cortisol which may contribute along with metabolic factors, to cognitive impairment. Finally, it was observed that differences in the shape of the diurnal cortisol profiles were attributable to various psychological and metabolic characteristics. Flattened profiles (non-classic), which have been associated with non-compliance, were associated with greater subjective reporting of stress, intensity of daily hassles, poorer sleep quality and more severe metabolic syndrome symptomology. This highlights the usefulness of the basal profile in determining individual vulnerability to stress and poorer health. In conclusion, diurnal cortisol, central obesity and markers of metabolic syndrome may interact to influence hippocampal memory function.

## TABLE OF CONTENTS

<b>CHAPTER ONE: LITERATURE REVIEW</b>	<b>1</b>
<u>1.1 Stress</u>	<u>1</u>
1.1.1 Psychological Approaches to Stress	1
1.1.2 Biological Systems in the Stress Response	2
1.1.3 Allostasis & Allostatic Load	4
<u>1.2 The Fundamentals of Cortisol Activity</u>	<u>5</u>
1.2.1 What is Cortisol?	5
<u>1.3 Basal Circadian Activity of Cortisol</u>	<u>6</u>
1.3.1 Cortisol Awakening Response (CAR)	6
1.3.2 Factors related to the Cortisol Awakening Response	7
<u>1.4 Evaluating Stress Induction Techniques</u>	<u>12</u>
1.4.1 Stress Responsivity	13
1.4.2 Stress Habituation	16
<u>1.5 Cortisol, Central Obesity &amp; the Metabolic Syndrome</u>	<u>17</u>
1.5.1 Central Obesity: Definitions & Diagnosis	17
1.5.2 The Metabolic Syndrome: Definitions & Diagnosis	18
1.5.3 Cortisol & Central Obesity	19
1.5.3.1 Glucocorticoid Level in Central Obesity	20
1.5.3.2 The Cortisol Awakening Response and Cortisol Profile in the Centrally Obese	22
1.5.3.3 Stress Exposure & Cortisol in the Centrally Obese	24
1.5.4 Stress & the Metabolic Syndrome	27
1.5.5 Additional Factors influencing Cortisol in the Centrally Obese	28
<u>1.6 Cortisol &amp; Cognitive Performance</u>	<u>30</u>
1.6.1 Mechanisms of Action: MR & GR Receptors & the Hippocampus	31
1.6.2 Effects of Endogenous Cortisol on Memory	33
1.6.3 Effects of Exogenous Cortisol on Memory	34
1.6.4 Influence of Emotion	38
1.6.5 Gender Differences in the Cortisol-Cognition Relationship	38
1.6.6 Dehydroepiandrosterone (DHEA), Cortisol & Cognition	39
1.6.7 Cognitive Performance in the Centrally Obese	41
<u>1.7 Summary</u>	<u>42</u>
<b>CHAPTER TWO: AIMS &amp; OBJECTIVES</b>	<b>43</b>
<u>2.1 General Aims</u>	<u>43</u>
<u>2.2 Basal Cortisol in High/Low Waist-Hip Ratio (WHR) Individuals</u>	<u>43</u>
2.2.1 Reproducibility & Consistency of Cortisol Diurnal Profiles	43
2.2.2 Patterns of Basal Diurnal Cortisol in High/Low WHR Individuals in relation to Psychological & Metabolic Parameters	44
<u>2.3 Cortisol responses to Stress in High/Low WHR Individuals</u>	<u>45</u>
<u>2.4 The effect of Psychological Stress on Cognitive Performance in High/Low     WHR Individuals</u>	<u>45</u>
<b>CHAPTER THREE: GENERAL METHODOLOGY</b>	<b>46</b>
<u>3.1 Introduction</u>	<u>46</u>
<u>3.2 Measurement of Obesity</u>	<u>46</u>
3.2.1 Body Mass Index (BMI)	46
3.2.2 Body Composition	47
3.2.3 Waist-Hip Ratio (WHR)	47

3.2.3.1 Obesity Related Biomarkers	49
<b>3.3 Measurement of Cortisol</b>	<b>53</b>
3.3.1 Cortisol	53
3.3.2 Dehydroepiandrosterone (DHEA)	57
3.3.3 Assessing Cortisol Diurnal Profiles (Computational Indices)	57
<b>3.4 Biopsychological Stress Induction &amp; Measurement</b>	<b>59</b>
3.4.1 Stress Induction: The Trier Social Stress Test	59
3.4.2 Subjective Measures of Stress	61
3.4.2.1 Perceived Stress Scale (PSS)	61
3.4.2.2 Daily Hassles Scale	62
3.4.2.3 Visual Analogue Scales (Debrief Questionnaire)	62
<b>3.4.3 Additional Physiological Measures of Stress</b>	<b>63</b>
3.4.3.1 Blood Pressure	63
<b>3.5 Measuring Cognitive Performance</b>	<b>63</b>
3.5.1 Background	64
3.5.2 Cognitive Tasks	64
3.5.3 National Adult Reading Test (NART)	68
<b>3.6 Conclusions</b>	<b>69</b>

#### **CHAPTER FOUR: THE PRACTICALITIES OF ASSESSING BASAL SALIVARY CORTISOL: A PILOT STUDY**

	<b>70</b>
<b>4.1 Introduction</b>	<b>70</b>
<b>4.2 Objectives</b>	<b>71</b>
<b>4.3 Method</b>	<b>71</b>
4.3.1 Sample	71
4.3.2 Design	71
4.3.3 Measures	71
4.3.3.1 Psychological Measures	71
4.3.3.2 Physiological Measures	72
4.3.3.3 Biological Measures (Cortisol)	72
4.3.4 Procedure	72
4.3.5 Data Treatment & Analysis	73
<b>4.4 Results</b>	<b>74</b>
4.4.1 Sample Characteristics	74
4.4.2 Diurnal Profiles	74
<b>4.5 Discussion</b>	<b>81</b>
4.5.1 Inter- & Intra- Subject Variability	82
4.5.2 Methodological Issues	83
<b>4.6 Conclusions</b>	<b>85</b>

#### **CHAPTER FIVE: BASAL CORTISOL VARIABILITY IN OLDER ADULTS: EXPLORING ASSOCIATED BIOPSYCHOLOGICAL PARAMETERS**

	<b>86</b>
<b>5.1 Introduction</b>	<b>86</b>
<b>5.2 Objectives</b>	<b>87</b>
<b>5.3 Method</b>	<b>88</b>
5.3.1 Sample	88
5.3.2 Design	89
5.3.3 Measures	90
5.3.3.1 Psychological Measures	90
5.3.3.2 Biological Measures	92

5.3.4 Procedure	92
5.3.4.1 Cortisol Monitoring Period	92
5.3.5 Data Treatment & Analysis	94
5.3.5.1 Missing Data	94
5.3.5.2 Data Screening	95
5.3.5.3 Statistical Analysis	95
<b>5.4 Results</b>	<b>97</b>
5.4.1 Sample Characteristics	97
5.4.2 Consistency of the Basal Diurnal Profile	98
5.4.3 Basal Cortisol in High/Low WHR Males/Females	99
5.4.3.1 Indices of Cortisol in High/Low WHR Males/Females	100
5.4.4 Classifying Basal Profiles	101
5.4.4.1 Basal Cortisol in, High /Low Waist-hip Ratio, Gender & Classic/Non-Classic Profile groups	102
5.4.4.2 Sleep Quality	103
5.4.4.3 Effect of Waking Time on the Basal Cortisol Profile	106
5.4.4.4 Daily Hassles & Perceived Stress	109
5.4.4.5 Blood Biomarkers & DHEA	112
5.4.5 Summary of Results	120
<b>5.5 Discussion</b>	<b>120</b>
5.5.1 General Aims	120
5.5.2 Basal Salivary Cortisol in High/Low WHR Males/Females	121
5.5.2.1 Differences in Basal Cortisol	121
5.5.2.2 Biomarkers of Obesity & Metabolic Syndrome	122
5.5.2.3 Cortisol & Perceived Stress/Daily Hassles	124
5.5.3 Classic & Non-Classic Cortisol Diurnal Profiles	125
5.5.3.1 Metabolic Syndrome & the Basal Cortisol Profile	125
5.5.3.2 Influence of Sleep on the Basal Cortisol Profile	127
5.5.3.3 Influence of Daily Hassles on the Basal Cortisol Profile	127
5.5.3.4 Influence of Waking Time on the Basal Cortisol Profile	127
5.5.4 Methodological Issues & Study Limitations	129
5.5.4.1 Compliance & the Basal Cortisol Profile	129
<b>5.6 Conclusions</b>	<b>130</b>
<b><u>CHAPTER SIX: EXPLORING STRESS RESPONSIVITY IN THE CENTRALLY OBESE USING THE TRIER SOCIAL STRESS TEST (TSST): IMPACT ON COGNITIVE FUNCTION USING CANTAB</u></b>	
<b>6.1 Introduction</b>	<b>132</b>
<b>6.2 Objectives</b>	<b>133</b>
<b>6.3 Method</b>	<b>133</b>
6.3.1 Sample	133
6.3.2 Design	134
6.3.3 Measures	134
6.3.3.1 Psychological Measures	134
6.3.3.2 Biological Measures	135
6.3.4 Procedure	136
6.3.4.1 Monitoring Day One	136
6.3.4.2 Test Day	136
6.3.4.3 Monitoring Day Two	138
6.3.5 Data Treatment & Analysis	139
6.3.5.1 Missing Data	139

6.3.5.2 Data Screening	139
6.3.5.3 Statistical Analysis	139
<b>6.4 Results</b>	<b>141</b>
6.4.1 Sample Characteristics	141
6.4.2 Stress Responsivity, Central Obesity & Cognitive Performance	143
6.4.2.1 Salivary Cortisol Response to the Stress/No Stress Tasks in High/Low WHR	143
6.4.2.2 Calculated Cortisol Response to the Stress/No Stress Tasks (Percentage Rise)	145
6.4.2.3 Cortisol Change from Baseline (Recovery)	147
6.4.2.4 Blood Pressure Response to the Stress/No Stress Tasks in High/Low WHR Males/Females	148
6.4.2.5 Calculated BP Response to the Stress/No Stress Tasks in High/Low WHR Males/Females	152
6.4.2.6 Change from Baseline Blood Pressure (Anticipation & Recovery)	152
6.4.2.7 STAI & SSES Scores in High/Low WHR Males/Females by Stress/No Stress condition	153
6.4.2.8 Cognitive Performance in High/Low WHR Males/Females by Stress/No Stress condition	156
6.4.2.9 Cognitive Performance in Responders/Non-Responders	167
6.4.3 Associated Psychological & Biological Parameters	179
6.4.3.1 Subjective Evaluation in High/Low WHR Males/Females by Stress/No Stress condition	179
6.4.3.2 Basal Cortisol in High/Low WHR Males/Females	183
6.4.3.3 Classic & Non-Classic Diurnal Cortisol Profiles	185
6.4.4 Summary of Results	193
<b>6.5 Discussion</b>	<b>194</b>
6.5.1 General Aims	194
6.5.2 Stress Responsivity in High/Low WHR Males/Females	194
6.5.2.1 Physiological & Psychological Responses to Stress	194
6.5.2.2 Efficacy of the TSST	197
6.5.3 Cognitive Performance by Condition & Stress Responses	198
6.5.4 Basal Cortisol in High/Low WHR Males/Females	202
6.5.4.1 Basal Cortisol	202
6.5.4.2 Metabolic Parameters & the Basal Cortisol Profile	203
6.5.5 Methodological Issues & Study Limitation	204
<b>6.6 Conclusions</b>	<b>206</b>
<b>CHAPTER SEVEN: GENERAL DISCUSSION</b>	<b>208</b>
<b>7.1 General Aims</b>	<b>208</b>
<b>7.2 Basal Cortisol in High/Low WHR Individuals</b>	<b>208</b>
7.2.1 Reproducibility & Consistency of the Cortisol Profile	208
7.2.2 Patterns of Basal Cortisol in High/Low WHR Individuals in Relation to Psychological & Metabolic Parameters	210
<b>7.3 Cortisol Responses to Stress in High/Low WHR Individuals</b>	<b>214</b>
<b>7.4 The Effect of Psychological Stress on Cognitive Performance in High/Low WHR</b>	<b>216</b>
<b>7.5 Methodological Issue &amp; Limitations</b>	<b>220</b>
7.5.1 The Assessment of Basal Cortisol in the Centrally Obese	220
7.5.2 The Efficacy of the TSST	220



7.5.3 Assessing Cognitive Performance	221
7.5.4 Compliance & Basal Cortisol Profiling	222
7.6 Future Research	223
7.7 Concluding Remarks	225

---

<b>REFERENCES</b>	<b>226</b>
-------------------	------------

---

<b>APPENDIX</b>	<b>267</b>
-----------------	------------

<b>Appendix 1:</b> Volunteer Screening Booklet	
<b>Appendix 2:</b> Telephone Screening Questionnaire	
<b>Appendix 3:</b> Saliva Sampling Guide 1	
<b>Appendix 4:</b> Saliva Sample Guide 2	
<b>Appendix 5:</b> Salivette Instruction Sheet	
<b>Appendix 6:</b> Physiological Data Record Sheet	
<b>Appendix 7:</b> Colour Blindness Test	
<b>Appendix 8:</b> Subjective Evaluation Questionnaire	
<b>Appendix 9:</b> Post Stress Study Debrief Sheet	
<b>Appendix 10:</b> Perceived Stress Scale (PSS)	
<b>Appendix 11:</b> Leeds Sleep Evaluation Questionnaire (LSEQ)	
<b>Appendix 12:</b> Hospital Anxiety & Depression Scale (HADS)	
<b>Appendix 13:</b> Dutch Eating & Behaviour Scale (DEBQ)	
<b>Appendix 14:</b> National Adult Reading Test (List of words)	
<b>Appendix 15:</b> Daily Hassles Scale with Self-Report Section	
<b>Appendix 16:</b> Auditory Verbal Learning Task (List of words)	
<b>Appendix 17:</b> Table of F-values for the Cortisol Indices (Chapter Five)	
<b>Appendix 18:</b> State Trait Anxiety Inventory (STAI)	
<b>Appendix 19:</b> State Self Esteem Scale (SSES)	
<b>Appendix 20:</b> Table of F-values for Cognitive Performance by Condition	
<b>Appendix 21:</b> Table of F-values for Cognitive Performance by Response Group (a) cortisol and (b) stress response groups	
<b>Appendix 22:</b> Cortisol secreted across the test session from alternative baseline in High/Low WHR Males/Females (Means $\pm$ SEM)	

## LIST OF TABLES

<b>Table 4.1</b>	Sample Characteristics displaying age, BMI, WHR & Perceived Stress with Means $\pm$ SD	74
<b>Table 4.2</b>	Associated Psychological & Physiological Characteristics for Volunteer's 001 & 002 to accompany the Basal Cortisol Profile	79
<b>Table 4.3</b>	Associated Psychological & Physiological Characteristics for Volunteer's 003 & 004 to accompany the Basal Cortisol Profile	79
<b>Table 4.4</b>	Associated Psychological & Physiological Characteristics for Volunteer's 005 & 006 to accompany the Basal Cortisol Profile	79
<b>Table 4.5</b>	Associated Psychological & Physiological Characteristics for Volunteer's 007 & 008 to accompany the Basal Cortisol Profile	79
<b>Table 4.6</b>	Correlations for consistency across 3 sampling days for each cortisol index	81
<b>Table 5.1</b>	Sample Characteristics displaying age, BMI, WHR & Perceived Stress with Means $\pm$ SD	98
<b>Table 5.2</b>	Correlations for consistency across 3 sampling days for each cortisol index	98
<b>Table 5.3</b>	Summary of the Biological and Psychological Characteristics of a Classic & a Non-Classic Diurnal Profile	103
<b>Table 5.4</b>	Correlations for consistency across 3 sampling days for Waking Time	106
<b>Table 6.1</b>	Sample Characteristics displaying age, BMI, WHR, Estimated IQ & Perceived Stress with Means $\pm$ SD	142
<b>Table 6.2</b>	% Rise in Cortisol post stressor in High/Low WHR Males/Females (Means $\pm$ SD)	145
<b>Table 6.3</b>	% Rise in Systolic Blood Pressure post stressor in High/Low WHR Males/Females (Means $\pm$ SD)	152
<b>Table 6.4</b>	% Rise in Diastolic Blood Pressure post stressor in High/Low WHR Males/Females (Means $\pm$ SD)	152
<b>Table 6.5</b>	Frequency Table of Cortisol Responders & Non-Responders to stress induction	168
<b>Table 6.6</b>	Frequency Table of Stress Responders (Cortisol & Blood Pressure) & Non-Responders to stress induction	169

<b>Table 6.7</b>	Summary of the Biological and Psychological Characteristics of a <u>Classic &amp; a Non-Classic Diurnal Profile</u>	<u>186</u>
<b>Table 6.8</b>	Summary of Blood Biomarkers in High/Low WHR <u>Males/Females (Means <math>\pm</math> SD)</u>	<u>187</u>

## LIST OF FIGURES

<b>Figure 1.1</b>	<u>Diagrammatic Representation of the HPA Axis</u>	3
<b>Figure 1.2</b>	<u>Example of a typical Basal Cortisol Diurnal Profile</u>	6
<b>Figure 1.3</b>	<u>Adaptation of the Inverted ‘U’ Hypothesis of Stress-Induced Cognitive Impairment</u>	31
<b>Figure 3.1</b>	<u>Screen shot of the Motor Screening Task (CANTAB)</u>	65
<b>Figure 3.2</b>	<u>Screen shot of the Delayed Matching to Sample Task (CANTAB)</u>	65
<b>Figure 3.3</b>	<u>Screen shot of the Paired Associates Learning Task (CANTAB)</u>	66
<b>Figure 3.4</b>	<u>Screen shot of the Pattern Recognition Task (CANTAB)</u>	66
<b>Figure 3.5</b>	<u>Screen shot of the Spatial Recognition Memory Task (CANTAB)</u>	67
<b>Figure 3.6</b>	<u>Screen shot of the Rapid Visual Processing Task (CANTAB)</u>	67
<b>Figure 3.7</b>	<u>Screen shot of the Spatial Working Memory Task (CANTAB)</u>	68
<b>Figure 3.8</b>	<u>Screen shot of the Stockings of Cambridge Task (CANTAB)</u>	68
<b>Figure 4.1</b>	<u>Composite Diurnal Cortisol Profile for the sample (Means <math>\pm</math> SEM)</u>	74
<b>Figure 4.2</b>	<u>3 day Basal Diurnal Cortisol Profile for Volunteer 001</u>	75
<b>Figure 4.3</b>	<u>3 day Basal Diurnal Cortisol Profile for Volunteer 002</u>	75
<b>Figure 4.4</b>	<u>3 day Basal Diurnal Cortisol Profile for Volunteer 003</u>	76
<b>Figure 4.5</b>	<u>3 day Basal Diurnal Cortisol Profile for Volunteer 004</u>	76
<b>Figure 4.6</b>	<u>3 day Basal Diurnal Cortisol Profile for Volunteer 005</u>	77
<b>Figure 4.7</b>	<u>3 day Basal Diurnal Cortisol Profile for Volunteer 006</u>	77
<b>Figure 4.8</b>	<u>3 day Basal Diurnal Cortisol Profile for Volunteer 007</u>	78
<b>Figure 4.9</b>	<u>3 day Basal Diurnal Cortisol Profile for Volunteer 008</u>	78
<b>Figure 5.1</b>	<u>Flow Chart to illustrate the main study design with WHR &amp; gender as between subjects factors</u>	89
<b>Figure 5.2</b>	<u>Flow Chart to illustrate the Classic versus Non-Classic study design with WHR, gender &amp; Profile Group as between subjects factors</u>	90
<b>Figure 5.3</b>	<u>Flow chart to detail the stages of volunteer exclusion &amp; retention</u>	95
<b>Figure 5.4</b>	<u>Basal Diurnal Cortisol Profiles in high/low WHR males/females (Means <math>\pm</math> SEM)</u>	99
<b>Figure 5.5</b>	<u>Basal Diurnal Cortisol Profiles in high/low WHR (Means <math>\pm</math> SEM)</u>	100
<b>Figure 5.6</b>	<u>Cortisol Diurnal Profiles by the Classic/Non-Classic Profile Groups (Means <math>\pm</math> SEM)</u>	102
<b>Figure 5.7</b>	<u>Scores on the LSEQ by the Classic/Non-Classic Profile Groups (Means <math>\pm</math> SEM)</u>	104

<b>Figure 5.8</b>	Basal Cortisol Diurnal Profile in early & later risers (Waking Group) (Means $\pm$ SEM)	107
<b>Figure 5.9</b>	Perceived Stress Score in High/Low WHR males/females & by Classic/Non-Classic Profile Group (Means $\pm$ SEM)	111
<b>Figure 5.10</b>	Perceived Stress Score in High/Low WHR Profile Group & by Classic/Non-Classic Profile Group (Means $\pm$ SEM)	112
<b>Figure 5.11</b>	Fasting Plasma Insulin Score in High/Low WHR Profile Group & by Classic/Non-Classic Profile Group (Means $\pm$ SEM)	113
<b>Figure 5.12</b>	C-Reactive Protein Score in High/Low WHR Profile Group & by Classic/Non-Classic Profile Group (Means $\pm$ SEM)	118
<b>Figure 6.1</b>	Flow Chart to illustrate the main study design WHR & gender as between subjects factors	134
<b>Figure 6.2</b>	Test Session Schedule of Events	137
<b>Figure 6.3</b>	Cortisol secreted across the test session in High/Low WHR Males/Females (Means $\pm$ SEM)	143
<b>Figure 6.4</b>	Mean Cortisol secreted during the test session in High/Low WHR Males/Females by Stress/No Stress Condition (Means $\pm$ SEM)	145
<b>Figure 6.5</b>	% Rise in Cortisol in High/Low WHR Males/Females by Stress/No Stress Condition (Means $\pm$ SEM)	146
<b>Figure 6.6</b>	Recovery of Cortisol activity in High/Low WHR Males/Females by Stress/No Stress Condition (Means $\pm$ SEM)	147
<b>Figure 6.7</b>	Systolic Blood Pressure Responses by Stress/No Stress Condition (Means $\pm$ SEM)	149
<b>Figure 6.8</b>	Systolic Blood Pressure Responses in High/Low WHR Males/Females (Means $\pm$ SEM)	149
<b>Figure 6.9</b>	Diastolic Blood Pressure Responses in High/Low WHR Males/Females (Means $\pm$ SEM)	151
<b>Figure 6.10</b>	STAI Scores in High/Low WHR Males/Females by Stress/No Stress Condition (Means $\pm$ SEM)	154
<b>Figure 6.11</b>	SSES Scores in High/Low WHR Males/Females by Stress/No Stress Condition (Means $\pm$ SEM)	156
<b>Figure 6.12</b>	Mean number of words recalled on Trials 1-5 of the AVLT by Stress/No Stress condition (Means $\pm$ SEM)	158
<b>Figure 6.13</b>	Mean number of words recalled from Trials 1-5 of the AVLT in	

	<u>High/Low WHR Males/Females (Means <math>\pm</math> SEM)</u>	158
<b>Figure 6.14</b>	Total number of words recalled from List A in High/Low WHR Males/Females (Means $\pm$ SEM)	160
<b>Figure 6.15</b>	DMS Error Probability in High/Low WHR Males/Females by Stress/No Stress Condition (Means $\pm$ SEM)	162
<b>Figure 6.16</b>	Total number of correct trials on the paired associates learning task (PAL) in High/Low WHR by Stress/No Stress condition (Means $\pm$ SEM)	163
<b>Figure 6.17</b>	%Correct on the spatial recognition task (SRM) in High/Low WHR Males/Females by Stress/No Stress Condition (Means $\pm$ SEM)	165
<b>Figure 6.18</b>	DMS Error Probability in High/Low WHR Males/Females Cortisol responders (Means $\pm$ SEM)	172
<b>Figure 6.19</b>	DMS Error Probability in High/Low WHR Males/Females Stress responders (combined cortisol & BP) (Means $\pm$ SEM)	173
<b>Figure 6.20</b>	Total number of correct trials on the paired associates learning task (PAL) in High/Low WHR Males/Females Stress responders (combined cortisol & BP) (Means $\pm$ SEM)	174
<b>Figure 6.21</b>	%Correct on the spatial recognition task (SRM) in High/Low WHR Males/Females cortisol responders (Means $\pm$ SEM)	175
<b>Figure 6.22</b>	%Correct on the spatial recognition task (SRM) in High/Low WHR Males/Females stress responders (combined cortisol & BP) (Means $\pm$ SEM)	176
<b>Figure 6.23</b>	Mean Initial Thinking time on the Stockings of Cambridge Task (SOC) in High/Low WHR Males/Females stress responders (combined cortisol & BP) (Means $\pm$ SEM)	178
<b>Figure 6.24</b>	Subjective Task Evaluation of the stress & cognitive tasks in High/Low WHR Males/Females by Stress/No Stress Condition (Means $\pm$ SEM)	179
<b>Figure 6.25</b>	Subjective Task Evaluation of the Stress/No Stress & Cognitive tasks (Perceived Stress) Females by Stress/No Stress Condition (Means $\pm$ SEM)	180
<b>Figure 6.26</b>	Subjective Task Evaluation of the Stress/No Stress & Cognitive tasks (Performance) in High/Low WHR	

	<u>(Means <math>\pm</math> SEM)</u>	182
<b>Figure 6.27</b>	Basal Diurnal Cortisol Profiles in High/Low WHR	
	<u>(Means <math>\pm</math> SEM)</u>	183
<b>Figure 6.28</b>	Basal Diurnal Cortisol Profiles in the Classic/Non-Classic	
	<u>Profile Groups (Means <math>\pm</math> SEM)</u>	185
<b>Figure 6.29</b>	Fasting Plasma Insulin in High/Low WHR males/females	
	<u>&amp; by Classic/Non-Classic Profile Group (Means <math>\pm</math> SEM)</u>	187
<b>Figure 6.30</b>	Fasting Glucose in High/Low WHR males/females	
	<u>&amp; by Classic/Non-Classic Profile Group (Means <math>\pm</math> SEM)</u>	189
<b>Figure 6.31</b>	Insulin Resistance in High/Low WHR males/females	
	<u>&amp; by Classic/Non-Classic Profile Group (Means <math>\pm</math> SEM)</u>	190

**ABBREVIATIONS (arranged thematically)**

<b>WHR</b>	Waist-Hip Ratio
<b>BMI</b>	Body Mass Index
<b>WSR</b>	Waist-to-Stature Ratio
<b>GAS</b>	General Adaptation Model of Stress
<b>HPA</b>	Hypothalamic-Pituitary-Adrenal Axis
<b>CNS</b>	Central Nervous System
<b>PNS</b>	Peripheral Nervous System
<b>ANS</b>	Autonomic Nervous System
<b>SNS</b>	Somatic Nervous System
<b>ACTH</b>	Adrenocorticotrophin
<b>CRH/CRF</b>	Corticotrophin Releasing Hormone/Factor
<b>SCN</b>	Suprachiasmatic Nucleus
<b>ATP</b>	Adenosine Triphosphate
<b>CA1</b>	Cornu Ammonis Region 1
<b>APR</b>	Acute Phase Response
<b>MR</b>	Mineralcorticoid Receptor
<b>GR</b>	Glucocorticoid Receptor
<b>NMDA</b>	N-methyl-D-aspartate receptors
<b>CBG</b>	Corticosteroid Binding Globulin
<b>11<math>\beta</math>-HSD</b>	11 $\beta$ -Hydroxysteroid Dehydrogenase
<b>NPY</b>	Neuropeptide-Y
<b>LTP</b>	Long Term Potentiation
<b>LTD</b>	Long Term Depotentiation
<b>DHEA</b>	Dehydroepiandrosterone
<b>DHEAS</b>	Dehydroepiandrosterone – Sulphate
<b>DEX</b>	Dexamethasone
<b>NK Cells</b>	Natural Killer Cells
<b>REM</b>	Rapid Eye Movement Sleep
<b>EEG</b>	Electroencephalography
<b>MS</b>	Metabolic Syndrome
<b>IL-6</b>	Interleukin-6
<b>CRP</b>	C-Reactive Protein



<b>HDL</b>	High Density Lipoprotein
<b>LDL</b>	Low Density Lipoprotein
<b>HOMA</b>	Homeostasis Model Assessment
<b>IGT</b>	Impaired Glucose Tolerance
<b>RIA</b>	Radioimmunoassay
<b>EIA</b>	Enzyme Immunoassay
<b>CAR</b>	Cortisol Awakening Response
<b>AUC</b>	Area under the Curve (with reference to 0)
<b>AURC</b>	AUC with reference to Sample 1
<b>BP</b>	Blood Pressure
<b>TSST</b>	Trier Social Stress Test
<b>CANTAB</b>	Cambridge Automated Neuropsychological Test Battery
<b>AVLT</b>	Auditory Verbal Learning Task
<b>PAL</b>	Paired Associates Learning
<b>SRM</b>	Spatial Recognition Memory
<b>SWM</b>	Spatial Working Memory
<b>RVP</b>	Rapid Visual Processing
<b>PRM</b>	Pattern Recognition Memory
<b>SOC</b>	Stockings of Cambridge
<b>MOT</b>	Motor Screening
<b>DMS</b>	Delayed Matching to Sample
<b>DEBQ</b>	Dutch Eating Behaviour Questionnaire
<b>LSEQ</b>	Leeds Sleep Evaluation Questionnaire
<b>HADS</b>	Hospital Anxiety and Depression Scale
<b>STAI</b>	State Trait Anxiety Inventory
<b>SSES</b>	State Self Esteem Scale
<b>NART</b>	National Adult Reading Test
<b>PSS</b>	Perceived Stress Scale

**PUBLICATIONS/PRESENTATIONS**

Lasikiewicz, N., Hendrickx, H., Talbot, D., & Dye, L (2003) Cortisol Variability & Lifestyle. Institute of Psychological Sciences, University of Leeds, Internal Postgraduate Conference.

Lasikiewicz, N., Hendrickx, H., Talbot, D., & Dye, L (2003) Inter & Intra-Subject Variability in basal cortisol & mood: Relationship to perceived stress & sleep quality. 34th Annual International Society for Psychoneuroendocrinology (ISPNE) Conference: Protective and Damaging Effects of the Biobehavioural Stress Response; Cognitive, Systemic and Clinical Aspects, New York City, USA.

Lasikiewicz, N., Hendrickx, H., Talbot, D., & Dye, L (2004) Cortisol & stress responsivity in relation to central obesity: Impact on Cognitive Performance. Institute of Psychological Sciences, University of Leeds, Internal Postgraduate Conference.

Lasikiewicz, N., Hendrickx, H., Talbot, D., & Dye, L (2005) Investigating Stress Induced memory impairment using CANTAB in relation to central obesity. BPS Psychobiology Section Annual Scientific Meeting, Low Wood, Lake District.

Lasikiewicz, N., Hendrickx, H., Talbot, D., & Dye, L (2006) Examination of Cortisol Responses to the Trier Social Stress Test (TSST) in the Centrally Obese & Subsequent Impact on Cognitive Performance. Unilever Internal Corporate Research Review.

Lasikiewicz, N., Hendrickx, H., Talbot, D., & Dye, L (2006) Cortisol & Cognitive Performance in Individuals with Central Obesity. 37th Annual International Society for Psychoneuroendocrinology (ISPNE) Conference: Hormones and Brain from Cloning to Clinic, Leiden, The Netherlands.

Lasikiewicz, N., Hendrickx, H., Talbot, D., & Dye, L (2006) Exploring Stress Responsivity in the Central Obese using the Trier Social Stress Test: Evidence for greater responsivity in high WHR males. 10<sup>th</sup> International Congress on Obesity (ICO), Sydney, Australia.

Lasikiewicz, N., Hendrickx, H., Talbot, D., & Dye, L (2006) Classifying Basal Cortisol Diurnal Profiles: Is it Just a Matter of Compliance? BPS Psychobiology Section Annual Scientific Meeting, Low Wood, Lake District

## CHAPTER ONE

### Literature Review

---

---

The aim of this thesis is to explore stress responsivity in an older adult sample in relation to central obesity and the impact of these factors on cognitive performance. This chapter considers the relevant literature upon which this research question is based.

The review begins with an introduction to the concept of psychosocial stress and the biological systems involved in producing and regulating a stress response. The review pays particular attention to the activity of the stress hormone cortisol and its role in the stress response. The basal activity of cortisol is explored, in addition to associated variables that interact with cortisol activity. These included; perceived stress, sleeping behaviour, immunity, gender, and age. The review briefly explores the methods implemented in stress research and the potential for response habituation. The focus subsequently turns to the role of cortisol in central obesity and in the expression of the metabolic syndrome. Finally, the potential for cortisol, central obesity, and metabolic syndrome symptomology to influence cognitive performance is examined.

#### 1.1 Stress

It is important to explore the fundamental stress response and the systems of regulation in order to appreciate the role of cortisol.

##### 1.1.1 Psychological Approaches to Stress

Stress arises when individuals perceive that they cannot adequately cope with the demands being made on them or with threats to their well-being (Lazarus, 1966). A stressor is a stimulus that can elicit a psychological or physiological response. This external stimulus can be physical (actual threat or danger) or psychological (job strain, loss of a loved one etc). The internal representation of this stressor contributes to an overall perception of the stressor and dictates whether a response will ensue. This can manifest in the form of mental strain or actual physical harm (depending on the

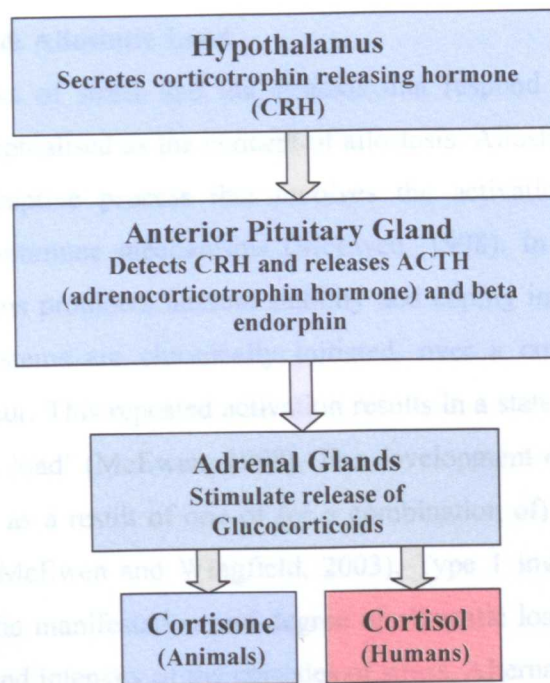
interpretation of the stressor for example, using past experience), emotional and defensive responses, and the initiation of coping strategies where necessary. These factors collectively determine the reaction of the body to the stressor i.e. the stress response. The response to a stressor is dependent on the psychological appraisal of the stimulus for example, is the stressor perceived as a threat? Dangerous? Harmful? If an individual copes effectively with stress exposure then little or no physiological stress response should be observed (Holroyd and Lazarus, 1982; Vogel, 1985). Similarly, if no stress response is observed then it is presumed that the individual is coping effectively (Levine, 1978).

Walter Cannon (1928) introduced the concept of 'homeostasis' or the maintenance of a stable internal environment. This concept views the response to stress as a regulatory system that allows itself to adapt in order to maintain stability. Cannon (1928) coined the phrase 'fight or flight' to account for the ability of the body to cope in emergencies by using one of two options; fight or flight in the response to a stressor. The 'fight or flight' approach formed the basis for Hans Selye's General Adaptation Model of stress (GAS) in the 1970's (Selye, 1974; 1979). This theory was the first to explore the response to stress from a biological and psychological perspective. The GAS model is based on four fundamental ideas. The first concerns the body's drive for stability of the internal system (homeostasis). The second concerns the ability of external stressors to disrupt the internal equilibrium of the body and induce a response. Third, is a period of resistance or adjustment to the stressor (which could be short or long). Fourth and finally, the body may reach exhaustion due to depleted energy resources and can no longer cope with the imposing stressor with potentially fatal consequences. This stage is not always reached but if energy resources are depleted then the organism may be more susceptible to illness and disease as energy resources are not replenished. The main biopsychological systems involved in the response to stress are outlined below.

### **1.1.2 Biological Systems in the Stress Response**

The human nervous system branches primarily into the central nervous system (CNS; brain and spinal cord) and the peripheral nervous system (PNS). The peripheral nervous system branches into two sub-components, the autonomic nervous system (ANS) and the somatic nervous system (SNS).

The ANS can be sub-divided into the sympathetic nervous system and the parasympathetic nervous system, both of which are implicated in the response to stress. The two-system view of the response to stress is based on the interaction between the HPA axis (hypothalamic-pituitary-adrenal axis) and ANS (Stanford and Salmon, 1993). The hypothalamus in the brain initiates the response through secretion of corticotrophin releasing factor as a signal to the anterior pituitary gland. The pituitary responds by releasing adrenocorticotrophin hormone (ACTH) and beta-endorphin (to mediate mood with analgesic properties). This change in endocrine status (ACTH) is detected by the adrenal glands and promotes the synthesis and release of glucocorticoids, corticosterone in animals and more importantly in humans, cortisol (Figure 1.1). Cortisol acts as the major chemical messenger in the HPA axis. Secreted in response to stress from the adrenal glands, cortisol is the key component in the negative feedback loop that regulates HPA activity. Elevated cortisol detected by the hypothalamus signals for a reduction in the activity of the response mechanism and thus can signal the end of the response.



**Figure 1.1:** Diagrammatic representation of the HPA Axis

There is evidence for the importance of the hippocampus in the feedback regulation of the HPA axis (Jacobsen and Sapolsky, 1991) due to the observation of the corticosteroid receptors; Type 1 (MR) and Type 2 (GR) receptors. Type 1 (MR) have a low affinity for glucocorticoids (cortisol) and only respond when cortisol is in low concentrations. MR

receptors are postulated to be important for the efficient regulation of the HPA axis. Type 2 receptors (GR) have a high affinity for glucocorticoids (cortisol) and respond when concentrations are high (De Kloet and Reul, 1987; Reul and De Kloet, 1985). Chronic stress can result in the over-activation of GR receptors which consequently overrides the effect of MR receptors when controlling the HPA axis.

In addition to the activation of the HPA axis, stressors also activate the sympathetic nervous system to increase the amount of available norepinephrine and epinephrine from the adrenal medulla. In times of stress, the body is prepared for action through stimulation of the mechanisms of priority for efficient coping. This results in an increase in the activity of the respiratory and cardiovascular systems whilst simultaneously reducing the activity of the gastrointestinal system and reproductive system. The hypothalamus secretes corticotrophin releasing factor as an alert to the adrenal glands to increase the production of the stress hormone epinephrine to allow for an increase in energy availability increasing stress responsiveness.

### **1.1.3 Allostasis & Allostatic Load**

Traditional views of stress and the systems that respond to stress have been more recently reconceptualised as the concept of allostasis. Allostasis defines the response to stress as an adaptive process that involves the activation of neuroendocrine and neuroendocrine-immune mechanisms (McEwen, 1998). In the short term, activating these mechanisms promotes internal stability and coping in the presence of a stressor. When these systems are chronically initiated, over a considerable period of time, damage can occur. This repeated activation results in a state of health which is referred to as 'allostatic load' (McEwen, 1998). The development of an 'allostatically loaded' state can occur as a result of one of (or a combination of) three experiences of stress dysregulation (McEwen and Wingfield, 2003). Type 1 involves frequent exposure to stress; hence, the manifestation and degree of allostatic load consequently depends on the frequency and intensity of the episodes of stress. Alternatively, a failure to cease the action of associated neural and endocrine response mechanisms can produce a state of allostatic load (Type 2) and finally, Type 3 involves a failure of the system to respond appropriately resulting in an under-response. These are all instances of failure within each associated system to adapt and as a result, subsequently increase the probability that stress related disease and impairments will result. Research highlights that a

primary factor in this outcome is the secretion of glucocorticoids (cortisol) (McEwen and Wingfield, 2003).

## **1.2 The Fundamentals of Cortisol Activity**

Cortisol is the primary output from the HPA axis and is the primary marker of stress responsivity. For this reason, this thesis focuses on the activity of cortisol as a marker of stress exposure and stress response in the centrally obese. The following sections explore the fundamentals of cortisol and related behaviours.

### **1.2.1 What is Cortisol?**

Cortisol is a corticosteroid hormone. Corticosteroids can be subdivided into mineralcorticoids e.g. aldosterone and glucocorticoids e.g. cortisol. Glucocorticoids are steroid hormones secreted by the adrenal glands, which chiefly promote the conversion of fats and proteins to glucose and glycogen for energy. Cortisol has a small molecular weight (362), is made from cholesterol, and is highly lipid soluble and poorly water-soluble. Research has identified glucocorticoid receptor sites in abundance in almost every cell of the body, enabling cortisol to have widespread effects. Hence, cortisol has the ability to influence in some way every major organ in the body and is responsible for normal organic and metabolic functioning (Munck et al., 1984). One of the ways in which this is achieved is through the hormonal regulation of gluconeogenesis. In times of stress, elevated cortisol levels potentiate the activity of the sympathetic nervous system and have a profound effect on glucose metabolism. The release of stored glucose and fats is increased and proteins are converted to increase energy availability. More specifically, cortisol signals for the breakdown of muscle protein leading to the release of amino acids into the bloodstream. Subsequently these amino acids are synthesised by the liver in order to synthesise glucose for energy in the process of gluconeogenesis (Miller and Tyrell, 1995). Cortisol is vital to the maintenance of normal functioning but at extremes (too little or too much) exposure to cortisol can have negative effects. Extremely elevated cortisol has been associated with blocking the action of insulin in taking up excess glucose which promotes the storage of energy in the form of fat. This fat is stored around the abdomen resulting in the development of central obesity which is accompanied by many problematic health consequences (Bjorntorp, 1997). Elevation of cortisol naturally results in an increase in gluconeogenesis, an elevated rate of



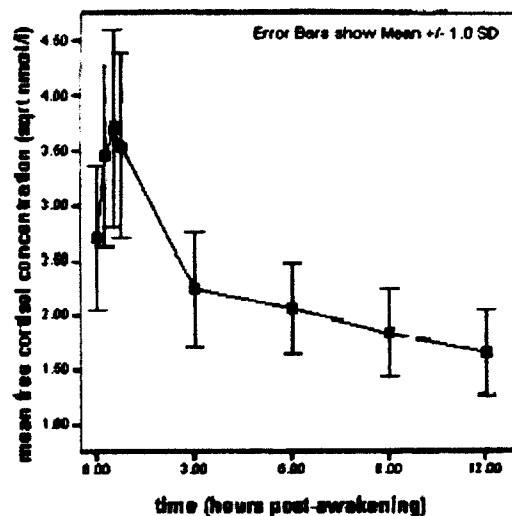
glucose production and ultimately the development of glucose intolerance concurrent with metabolic syndrome symptomology (Levitt et al., 2000) (see Section 1.5.2).

### 1.3 Basal Circadian Activity of Cortisol

Aside from stimulated activity, cortisol also exhibits a natural stable circadian rhythm comprising a cortisol awakening response and subsequent diurnal activity. This is discussed in the next section.

#### 1.3.1 Cortisol Awakening Response (CAR)

The transition from a sleeping state to an awakened state is characterised by a sharp burst in HPA activity indicated by increased ACTH and by elevations in cortisol secretion (Born et al., 1999). This response is initiated to prepare the body for the metabolic demands of the day in the transition from a rested state to an active phase (Prüssner et al., 1997). Cortisol commonly exhibits a 2-3 fold increase in most individuals during the first 30 to 45 minutes following waking (Edwards et al., 2001; Hucklebridge et al., 1998; Prüssner et al., 1997) before declining steadily to exhibit a stable diurnal profile (Figure 1.2). Cortisol demonstrates an average increase of 9nmol/l within a range of 4-15nmol/l (Clow et al., 2004) during the awakening response.



**Figure 1.2:** Example of a typical Basal Cortisol Diurnal Profile (taken from S.Edwards et al. / Life Sciences 68 (2001) 2093-2103)

Individuals can be characterised by their daily basal cortisol diurnal profile which can be associated with a number of factors, for example time of awakening (Edwards et al., 2001) and may also be predictive of general health (Roberts et al., 2004; Sapse, 1997; Smyth et al., 1997). Dettenborn et al. (2005) found that those at familial risk of breast cancer exhibited heritable cortisol responses to waking, whereas diurnal cortisol activity appeared not to be under genetic control. The cortisol awakening response has also been shown to be sensitive to factors such as burn out (Prüssner et al., 2003; See Section 1.3.2. part vi.), and chronic fatigue (Kudielka and Kirschbaum, 2003). Additional factors associated with the basal cortisol response to waking are discussed in the next section.

### **1.3.2 Factors related to the Cortisol Awakening Response:**

The cortisol response to waking may not be exclusively related to HPA activity. This section explores factors associated with the response and the extent of their influence.

#### **i. Blood Glucose**

As the cortisol awakening response is presumed to be a preparation process of the body for the metabolic demands of the day, it was initially hypothesised that an increase in awakening cortisol is both concurrent with and results from low fasted blood glucose (Bamberger et al., 1996). This was supported by the role of cortisol in the process of gluconeogenesis (Miller and Tyrell, 1995). Thus, it could be hypothesised that lower glucose levels at waking should result in a greater cortisol awakening response. However, Hucklebridge et al. (1999) found no association between the cortisol awakening response and blood glucose levels. It is clear that cortisol is closely associated with changes in blood glucose. However support for the role of blood glucose in the cortisol awakening response is not as well established as for the influence of blood glucose on cortisol responses to stress. A relationship between blood glucose and cortisol responses to the Trier Social Stress Test, a psychological stress induction technique (TSST, Kirschbaum et al., 1993: See Chapter Three, Section 3.4) has been demonstrated. Kirschbaum et al. (1997) found that glucose response positively correlated with cortisol responses during exposure to the TSST. In addition, elevation of blood glucose following a glucose load initiates a normal cortisol response to stress. Further, individuals in the low euglycaemic range (low blood glucose) often fail to initiate a substantial cortisol response when confronted with a stressor (Kirschbaum et

al 1997). However this does not concur with the relationship between blood glucose and the cortisol awakening response. Hucklebridge et al. (1999) found that individuals who had low blood glucose at waking were still capable of demonstrating a substantial cortisol response on waking. It appears then, that no correlation exists between cortisol awakening responses and stress responses (Schmidt-Reinwald et al., 1999) particularly when changes in blood glucose are considered. It is possible, therefore, that the cortisol awakening response is governed by a regulatory system that is distinct from that involved solely in HPA activity.

## **ii Waking time and Sleep**

Based on the hypothesis that the cortisol awakening response may not be solely governed by the HPA axis, research has suggested alternative systems that may exert some influence over the cortisol response to waking. One prominent system of interest is that which governs sleep, mediated largely by the Suprachiasmatic nucleus (SCN) in the hypothalamus, the same region that regulates the secretory activity of the pituitary and adrenal glands controlling ACTH and cortisol secretion (Van Cauter and Turek, 1995). This theory proposes that cortisol secretion is linked to the sleep-wake cycle. Research has highlighted the consistency between the circadian rhythmicity of ACTH and cortisol with patterns of sleep and waking in that cortisol is low during nocturnal sleep and the second half of nocturnal sleep is characteristic of increasing HPA activity (Weitzman et al., 1971). The activity of ACTH is consistent with this (Horrocks et al., 1990) and it has been suggested that the simultaneous increase in cortisol and ACTH combined with rapid eye movement sleep (REM) initiates spontaneous waking (Born et al., 1999; Spath-Schwalbe et al., 1992) followed immediately by the cortisol awakening response.

Cortisol and sleep are closely associated. Cortisol infusions during sleep can influence sleep patterns, affecting the amount of time spent in specific cycles of sleep (Born et al., 1989; Freiss et al., 2004). Born et al. (1989) observed that cortisol infusions decreased the amount of REM sleep. This is consistent with previous observations of lowered cortisol indicative of diminished adrenocortical activity during REM sleep (Follenius et al., 1992). Prinz et al. (2000) found that higher cortisol was associated with an earlier awakening time and less REM sleep in healthy older adults. Moreover, cortisol levels appear to be inhibited by slow wave sleep (Bierwolf et al., 1997; Brandenberger et al.,

1996; Scheen et al., 1996; Seifritz et al., 1995; Weitzman et al., 1983) and cortisol levels are stimulated through sleep disturbance (Vgontzas et al., 1999).

Sleep disturbance can influence cortisol activity on the subsequent day. For example, Backhaus et al. (2004) found a negative correlation between the cortisol awakening response and subjective rating of sleep quality. Lower cortisol immediately after waking correlated with a higher frequency of nightly awakenings and diminished sleep quality. However, the direct influence of sleep quality on the cortisol awakening response is under question and many studies have failed to find an effect. Hucklebridge et al. (2000) explored the effect of nocturnal waking on the subsequent cortisol awakening response and found no evidence of a direct impact. Similarly, Waye et al. (2004) found no effect of noise interruption during sleep on the subsequent cortisol awakening response. Some studies indicate that it is the remaining diurnal profile of cortisol that is most open to influence from such disturbances. For example, Leproult et al. (1997) found that partial sleep loss resulted in more elevated cortisol the following evening and suggested that sleep loss specifically affects HPA recovery during the circadian rhythm.

In addition to sleep quality, there is also evidence that the time of waking can influence subsequent cortisol activity in addition to (and often related to) sleep duration. There is evidence to suggest that individuals active in the morning hours demonstrate a greater elevation in salivary cortisol during the cortisol awakening response when compared to those active in the evening (Bailey and Heitkemper, 1991). Edwards et al. (2001) observed that early awakeners also exhibit more elevated cortisol levels throughout the remainder of the diurnal cycle despite showing a steeper decline when compared to late awakeners. Data concerning the duration of sleep was however, not collected in this study and whether those waking earlier experienced shorter sleep duration could not be ascertained. Some individuals can exhibit a more elevated cortisol awakening response from a shorter period of sleep (Spath-Schwalbe et al., 1992; Wüst et al., 2000b). However, Prüssner et al. (1997) failed to find any association between sleep duration and subsequent cortisol awakening responses.

It is clear that a close relationship exists between cortisol and patterns of sleep and waking. This relationship appears to be mediated via the Suprachiasmatic nucleus (SCN) in the hypothalamus. There is direct evidence that specific neural pathways

connect the SCN with the adrenal cortex (Buijs et al., 1993; 1997; 1998; 1999; Dijkswa et al., 1996; Kalsbeek et al., 1992; 1996). The SCN-HPA pathway may exert a regulatory influence over the cortisol awakening response in relation to patterns of sleep and waking. This is supported by the observed dissociation between cortisol and ACTH during the morning cortisol peak which implies that it is not simply the HPA axis that is involved (Born et al., 1999; Fehm et al., 1984; Spath-Schwalbe et al., 1991; Thorn et al., 2004). These findings contribute to the growing body of evidence which suggests that the cortisol awakening response and subsequent diurnal cortisol activity are independent (see Chapter Five, Section 5.5.3.4).

### **iii. Immune activity**

The cortisol awakening response has additionally been postulated to modulate the balance between cellular and humoral immunity. Cellular or Type 1 immunity involves 'cytotoxic' or cell destroying cells for intracellular organisms for example, viruses. Humoral or Type 2 immunity involves antibody secretion for extracellular organisms. (Petrovsky et al., 1997; Visser et al., 1998). Elevations in cortisol could mediate the change of night time Type 1 activity to daytime Type 2 activity (Edwards et al., 2003). Abnormal cortisol profiles have been associated with certain pathologies including cancer and AIDS (Sapsee, 1997). Flattened profiles (a blunted cortisol awakening response coupled with normal or elevated diurnal cortisol) have been found to be predictive of earlier mortality particularly in breast cancer patients (Sephton et al., 2000). These associations are presumed to occur as a result of suppressed anti-tumour cell activity of immune natural killer (NK) cells. However, Smyth et al. (1997) concluded that flattened profiles may be predictive of fewer pathological conditions. Thus, profiles of cortisol activity may reflect an individual's vulnerability and susceptibility rather than indicate their current health condition.

### **iv. Age**

Age is an important factor in shaping the cortisol awakening response and diurnal secretion profiles. Profiles become more flattened as a result of age (Wolf et al., 2002) and older adults tend to show more elevated overall cortisol secretion than younger adults (Nicolson et al., 1997). Profiles have been shown to become more inconsistent with age, with increased variation in the diurnal cycle. In many cases, the cortisol awakening response remains comparable to that in those of a younger age (Raff et al.,

1999) with the biggest changes observed in subsequent diurnal secretion. Otte et al. (2005) concluded from a meta-analysis that cortisol responses to challenge (for example, psychological stressors) increase with age and are more pronounced in females compared with males. However, this age related change is not always observed (Lupien et al., 1996). Research examining these proposed diurnal alterations is limited and therefore, this is in need of further assessment.

#### **v. Gender**

The evidence for gender differences in terms of basal circadian cortisol activity is conflicting. Some studies fail to observe a gender difference particularly in the cortisol awakening response (e.g. Kudielka and Kirschbaum, 2003). Some studies report a greater cortisol awakening response in middle-aged females compared to middle aged males (Scholtz et al., 2004; Schulz et al., 1998; Wright and Steptoe, 2005; Wüst et al., 2000). Others observe greater cortisol responses to waking (Prüssner et al., 1997) but only after thirty minutes with no difference in responses upon waking. Some studies report increased responsivity in males compared to females particularly in relation to stress exposure (Kirschbaum et al., 1992; Prinz et al., 2000). The main problem in exploring gender differences is that many studies are conducted in single gender samples which preclude such comparisons.

#### **vi. Perceived Chronic Stress & Burn Out**

The perception of stress correlates with changes in cortisol (Bernet et al., 1998; Lightman, 1995) but there is little evidence for perceived stress to be associated with changes in the cortisol response to waking. Goldman et al. (2005) found that older adults demonstrated a greater physiological response to perceived stress in terms of cortisol and certain biomarkers including IL-6, triglycerides, and fasted blood glucose. There was further evidence to suggest that these effects were more pronounced in older females compared with older males. However, the specific influence of this on the cortisol awakening response was not assessed.

Chronic exposure to stress can result in a state of 'burn out', a syndrome characterised by emotional exhaustion, physical fatigue, and mental weariness (Melamed et al., 1992; 1999; Shirom, 1989; 1997). Burnout has been associated with an altered cortisol awakening response but the literature conflicts in terms of how burnout specifically

alters cortisol secretion. In states of chronic burnout, cortisol profiles are flattened (Hellhammer, 1990; Melamed et al., 1999; Morgan et al., 2002; Prüssner et al., 1999). Yet, other studies have highlighted an elevated cortisol response to waking for example, Grossi et al. (2005) demonstrated elevated morning cortisol in females with high levels of burn-out compared with moderate and lower levels of burn-out identified by the Shirom-Melamed Burn-Out Questionnaire (SMBQ). This distinction was not so clear in males. Similarly, in an assessment of clinically diagnosed burnout patients, De Vente et al. (2003) demonstrated elevated morning cortisol responses. More recently, Mommersteeg et al. (2006) found no evidence of cortisol dysregulation in those with clinical burnout. Further research is clearly required. The observed differences in response patterns may be attributed to differences in the diagnosis of burnout and the severity of symptoms.

#### **1.4 Evaluating Stress Induction Techniques**

A number of different tools, techniques, and approaches have been implemented to induce a stress response so that stress related states can be assessed. Some stress induction tools have been more successful than others. The variance in response from such tools refutes the idea that stress responses are non-specific and all stressors, physical or psychological will elicit the same stress response (Selye, 1956). Some stress induction tools have failed to induce any change in cortisol (Manuck et al., 1991).

In a review of various stress induction tools, Dickerson and Kemeny (2004) identified a number of factors that are important in determining the effectiveness of stress induction. These are the specific contexts of the situation and include novelty (Rose, 1980), unpredictability (Mason, 1968), uncontrollability (Henry and Grim, 1990) and threat (Blascovich and Tomaka, 1996; Dienstbier, 1989). The differing stress induction tools were categorised in terms of their main characteristics in a meta-analysis (Dickerson and Kemeny, 2004). These classifications included: i) cognitive tasks, ii) public speaking/verbal interaction, iii) public speaking/cognitive combination, iv) noise exposure, v) emotional induction. In all, 208 techniques for stress induction were considered. The analysis revealed that tasks that included some form of social-evaluative threat in which others negatively judged performance and in which the outcome was uncontrollable proved the most reliable in inducing a significant stress

response. The inclusion of social-evaluative threat and uncontrollability affected response magnitude and rate of recovery. Public speaking tasks significantly elevated cortisol levels where noise exposure and emotional stress induction techniques failed to induce a significant cortisol response. The most effective stress induction tools were those that combined public speaking with a cognitive task counterpart. With this tool, the effect size was almost twice as large as for the remaining stress induction tools. Dickerson and Kemeny (2004) suggested that this particular tool was the most effective due to the combination of a form of social evaluative threat and uncontrollability. A good example of this type of task is the Trier Social Stress Test (TSST) (Kirschbaum et al., 1993), which combines a public speaking interview task with a mental arithmetic task in front of a panel of judges. Application of the TSST has been found to successfully elevate ACTH, cortisol (both serum and saliva), growth hormone, prolactin, and heart rate post administration (Kirschbaum et al., 1993). More specifically, a 2- to 4- fold increase in cortisol above baseline has been observed in just under 80% of subjects (Schommer et al., 2003) and a 2.5mmol/l increase in cortisol observed as a result of exposure (Kirschbaum et al., 1993). The TSST is a reliable tool for stress induction and is an important application in the study of stress responses.

Therefore, it appears that stress responses can be effectively and reliably induced in the laboratory using the right tools. Response to such challenges occur in most cases but can still be open to individual differences in terms of gender and age, both of which may significantly affect the process of habituation. It is those individuals, therefore, who respond most to such tools and who fail to habituate who are most at risk from developing stress related illness and disease.

#### **1.4.1 Stress Responsivity**

Studies of the biological response to stress are informative when determining an individual's vulnerability to the negative effects of stress. Individuals who show elevated stress responses to laboratory stressors are more at risk of developing a range of metabolic and cardiovascular related illness and disease including cardiovascular disease (Kirschbaum et al., 1995). There exists significant heterogeneity in the literature in terms of individual difference in the response to stress, in particular, the ability of one individual to habituate to a repeated stressor when another does not and what these differences can be attributed to. It has been suggested that these observed individual



differences in response are of paramount importance and essentially reflect the current state of the hypothalamic-pituitary-adrenal axis and its regulation (Keller-Wood and Dallman, 1984).

#### **i. The Influence of Cognitive Appraisal on the Response to Stress**

As discussed in Section 1.1.1, it was traditionally supposed that if an individual can cope effectively with stress exposure then little or no physiological stress response will be observed (Holroyd and Lazarus, 1982; Vogel, 1985). Similarly, if no stress responses/cortisol elevations are observed then it is presumed that the individual is coping effectively (Levine, 1978). Lazarus and Folkman (1984) suggested that cognitive appraisal was important to the likelihood that a stress response will occur. If an individual perceives a threat (primary appraisal) and further that the individual is unable to remove the threat (secondary appraisal) then the individual will experience a stress response. Indeed, cognitive appraisal of the Trier Social Stress Test has been shown to correlate with subsequent cortisol responses (Gaab et al., 2005) and that this has been shown to explain 35% of the variance in the cortisol response (Rohrmann et al., 1999). Based on this theory, research has explored in detail the use of effective coping strategies in determining the magnitude of a stress response in the experience of daily stressors. Bohnen et al. (1991) examined a variety of coping strategies that could be employed in response to a daily stressor and found that use of coping strategies was, in part, effective in maintaining minimal response initiation. The study found that by employing 'comforting cognitions' (from the Utrecht Coping List), considering the problem in a relative way, using self encouragement and reframing the problem in a positive way, served as powerful predictors of subsequent physiological response to a stressor. This has further been shown, to modulate the cortisol response to pharmacological activation of the HPA axis using pentagastrin (Abelson et al., 2006).

#### **ii. Age Differences in Stress Responsivity**

The literature provides conflicting evidence for changes in stress responsivity (both cortisol and ACTH) with advancing age. Gotthardt et al. (1995) found greater stress responsivity in older adults compared with younger adults participating in the same study. Lindheim et al. (1992) found greater responsivity in pre-menopausal females compared to post-menopausal females. Similarly, Kudielka et al. (2004) found that the ACTH response to a psychological stressor in young males decreased with advancing

age which suggests that adrenal sensitivity is greater in young males and decreases with age. However, Kudielka et al. (1999 and 2000) previously failed to identify any age related changes in either males or females. It would seem plausible that stress responses increase with age due to a higher basal level of glucocorticoids (e.g. Lupien et al., 1995). This is consistent with the findings reported by Gotthardt et al. (1995). Alternatively, the literature which explores glucocorticoid secretions with advancing age suggests that it would be plausible to expect a diminished response to stress due to down-regulation of glucocorticoid receptors from chronic life stress (see Lupien et al., 1994). The issue of age effects clearly merits further research.

### **iii. Gender Differences in Stress Responsivity**

Most research fails to report gender differences in response to stress (e.g. Kudielka et al., 2004) Others report only differences in younger populations (Collins and Frankenhauser, 1978; Forsman and Lundberg, 1982; Frankenhauser et al., 1978; Kirschbaum et al., 1992; 1995; Lundberg, 1983). Seeman et al. (1995) reported greater stress responsivity in elderly females compared to males. However, Wolf et al. (2001) found the opposite in a study designed to assess possible gender differences. They found that males demonstrated a more pronounced response to a stress induction task compared to females in their study which explored the effect of stress induced glucocorticoids on cognitive function. Kirschbaum et al. (1999) argue that the effect of gender is not evident in plasma cortisol levels but is evident in changes in ACTH levels and total free salivary cortisol (ACTH release is the stimulus for cortisol release from the adrenal glands). It appears that ACTH tends to be elevated in males compared to females regardless of menstrual cycle phase and/or oral contraceptive use. The observation that when gender differences are observed, males tend to be more responsive suggests that females are in some way protected from over-responding. Wolf et al. (2001) propose that this is due to the activity of sex hormones, particularly oestrogen, which may protect against the effect of stress (Galea et al., 1997). Differences in responsivity due to oestrogen could be linked to the activity of corticosteroid binding globulin (CBG; bound to the majority of circulating cortisol). CBG is higher in pre-menopausal females than in males of the same age (Fernandez-Real et al., 2003) and is higher during oral contraceptive use (Fujimoto et al., 1986; Weigartz et al., 2003). CBG synthesis is stimulated by oestrogen and so may explain the

reduced bioavailability of cortisol in females and the lesser response to psychological stressors (Kajantie and Phillips, 2006).

#### **iv. Other factors**

Other factors that have been explored to explain the individual differences that have been observed include genetic heritability yet there is little evidence to suggest genetic predisposition to stress responsivity (Wüst et al., 2005), however, polymorphisms in the glucocorticoid receptor gene have been associated with adrenocortical responses to stress (Wust et al., 2004). Further, exhaustion appears to correlate with a loss of stress response habituation (Kudielka et al., 2005). Finally, a low birth weight has been associated with elevated salivary cortisol responses to psychosocial stress (Wust et al., 2005). These are factors to consider when exploring individual difference in the response to a psychological stressor.

#### **1.4.2 Stress Habituation**

Differences in the ability to habituate to a repeated stressor have also been observed. In the long term, failure to habituate may predispose an individual to greater risk of stress related illness and disease. Kirschbaum et al. (1995) observed that some individuals fail to habituate to a repeated stressor. These 'high responders' demonstrated the same magnitude of cortisol elevation at each presentation of the stressor compared to 'low responders' who demonstrated an initial rise on the first exposure but failed to show an equivalent response on subsequent exposures. Schommer et al. (2003) found clear habituation of the HPA axis which did not arise from underlying sympathetic nervous system responses, evidenced by clear habituation of cortisol responses concurrent with a lack of change in norepinephrine and epinephrine secretion. Differences in habituation can be explained in the same way as gender and ageing influences due to a number of factors including the intensity, number and frequency of stress exposure as well as to individual experience of stress and coping. All of these factors determine the development of habituation to some degree (De Souza et al., 1986; Natelson et al., 1988; Pitman et al., 1988; 1990; Schommer et al., 2003; Terrazzino et al., 1995). However, separating these factors in terms of importance is difficult.

In summary, it is evident that cortisol is a key component of the stress response. Cortisol demonstrates a diurnal profile of basal activity and can be clearly measured in

response to stress using stress induction techniques. What is less well established is if these patterns of cortisol activity, both basal and in response to psychological stress differ in relation to the presence of central obesity and the metabolic syndrome. This will be discussed in the next section.

## **1.5 Cortisol, Central Obesity & the Metabolic Syndrome**

### **1.5.1 Central Obesity: Definitions & Diagnosis**

Obesity is categorised in terms of the distribution and type of fat present. Presence of fat specifically around the abdomen is termed 'central adiposity' (androgenoid physiognomy) as distinct from peripheral obesity or 'full body obesity' (gynaecoid proportions). Central obesity differs from peripheral obesity by the type of fatty tissue present. Central fat is marked by an increase in adipose tissue in the form of visceral fat. Visceral fat is more sensitive fatty tissue which has increased blood flow compared to other types of fatty tissue (for example, subcutaneous peripheral fat) and contains an increased number of glucocorticoid receptors. As a direct result of this, visceral fat is more sensitive to the presence and action of glucocorticoids such as cortisol and the action of triglycerides (Pedersen et al., 1994). Further, adipose tissue is a known target organ for glucocorticoids (Feldman, 1978). Research has shown that the maturation of human adipocyte precursor cells into mature fat cells is triggered by the presence of cortisol and insulin (Hauner et al., 1987). Therefore, exposure to elevated glucocorticoids, the presence of insulin and elevated blood lipids causes the tissue to respond by further accumulating fatty tissue and consequently increasing its size. Research has shown that there are more cells per unit mass in visceral fat compared to peripheral subcutaneous fat (Bjorntorp, 1995). Changes in the amount of visceral fatty tissue accumulated have been found to facilitate the release of free fatty acids into the blood stream and contribute to cholesterol synthesis, increasing the risk of developing insulin resistance (Bjorntorp, 1997).

The World Health Organisation (WHO, 2000) defines central obesity as a waist-hip ratio (WHR) of greater than 0.85 in females and 0.90 in males, a calculation based on accurate measurement of waist circumference compared to hip circumference. The presence of central obesity can also be identified by single measurement of waist circumference such that a measurement greater than thirty-five inches in females and

forty inches in males indicates central obesity (NCEP, 2001). Measurement of abdominal fat can also be determined from assessment of skin fold thickness using callipers (Durnin and Rahaman, 1967) or by use of bio-impedance (Kushner, 1992; Kotler et al., 1996). More recent research has utilised computerised tomography (Rockall et al., 2003) to determine the volume of visceral tissue exhibited for an individual in relation to their overall obesity status (e.g. body mass index, BMI). Some techniques have been found to be more accurate than others but each technique has contributed to the modern understanding of the diagnosis of central obesity and its implications.

The use of calculated waist-hip ratio as a tool has helped to develop understanding of the link between obesity status and health status (see Chapter Three, Section 3.2). Hartz et al. (1984) reported that individuals with a high waist-hip ratio also had a higher prevalence of diabetes and hypertension compared to those with a low waist-hip ratio. The risk of developing such disorders in those with central obesity rises from 3% to 10% regardless of the degree of obesity. Similar results were obtained using skin fold thickness as a diagnostic tool for central adiposity (Blair et al., 1984). Kalkhoff et al. (1983) identified a correlation between increase in central obesity and increases in blood pressure, insulin and reduction in carbohydrate tolerance. Indeed, waist-hip ratio and the diagnosis of central obesity appear more useful in identifying risk for insulin resistance and diabetes than weight alone (Rivera and Svec, 1989).

### **1.5.2 The Metabolic Syndrome: Definitions & Diagnosis**

The acknowledgment that central obesity is associated with a number of negative health consequences prompted the suggestion that central obesity is an important component of the metabolic syndrome. The metabolic syndrome is a cluster of metabolic and cardiovascular risk factors (Isomaa, 2003). These symptoms include; impaired glucose tolerance and/or insulin resistance, obesity (specifically, central obesity), dyslipidemia, and hypertension (WHO, 1999). The metabolic syndrome was first formally identified by Reaven in 1988 who suggested that insulin resistance and compensatory hyperinsulinemia were central to a cluster of symptoms that make up the metabolic syndrome. The degree of insulin resistance has since been identified as the key component of the syndrome and has been shown to be the important link between the syndromes main features (De Fronzo and Ferannini, 1991). The metabolic syndrome

occurs in approximately 15% of males and 10% females without prior impaired glucose tolerance and prevalence increases when there is pre-existing insulin resistance and diabetes mellitus to 84% of males and 78% of females (Isomaa et al., 2001). A number of theories have been proposed to explain the development of metabolic syndrome. These include individual lifestyle theories, for example, the amount of physical activity and exercise (Whaley et al., 1999), early prenatal experience, particularly low birth weight and reduced fetal growth (Jaquet et al., 2000) and also genetic theories based on the heritability of individual characteristics of the syndrome which comprise, obesity (Maes et al., 1997), type 2 diabetes (e.g. Groop et al., 1996), hypertension (e.g. Levy et al., 2000), elevated triglycerides and HDL cholesterol (Snieder et al., 1999) and cortisol (Bjorntorp and Rosmond, 2000) (See Section 1.5.4).

### **1.5.3 Cortisol & Central Obesity**

The presumed role of cortisol in the expression of obesity, specifically, central obesity and the metabolic syndrome is complex. Initial suggestions that a glucocorticoid excess was in some way related to visceral adiposity resulted from observations of patients with Cushing's syndrome (Bjorntorp and Rosmond, 2000). Cushing's syndrome is a condition of cortisol excess with many obvious characteristics, one of which is central obesity. It has been reported that patients with Cushing's syndrome demonstrate the same risk and tendency to develop insulin resistance, diabetes, dyslipidemia, hypertension, and risk of cardiovascular disease as those with a high waist-hip ratio (Bjorntorp and Rosmond, 2000). Researchers have attempted to ascertain whether cortisol plays a central role in the expression of central obesity and if it is in some way related to a dysfunctioning HPA axis. In addition, if cortisol is identified as a key factor in central obesity, the direction of the relationship could be that cortisol excess precedes the obese state or that the cortisol excess and associated negative health aspects are the result of pre-existing obesity. It is difficult to separate cause from effect because glucocorticoids have not been measured prior to the onset of central obesity in longitudinal studies (for a review see Bjorntorp and Rosmond, 2000). The vast majority of research in this area examines whether glucocorticoid excess is present in individuals whose central obesity is pre-established in comparison to those who do not exhibit visceral fat accumulation (peripheral) or lean individuals. Hence, these correlational studies do not clarify the direction of the relationship or the nature of cause and effect.

Obesity is a heterogeneous condition and the task of separating cause from consequence is a complex one.

### **1.5.3.1 Glucocorticoid Level in Central Obesity**

Historically, clinical studies have revealed that cortisol production in the obese is elevated (Simkin et al., 1961; Szenas and Pattee, 1959). However, the distribution of fat and type of fat exhibited was not considered by research until recently and early findings need to be treated with caution. It is also important to consider the possibility that the larger body of an obese individual could show more cortisol activity simply because of its mass (Rivera and Svec, 1989). Indeed, many researchers have highlighted the difficulty in comparing obese and non-obese individuals (Migeon et al., 1963; Streeten et al., 1969). In pregnancy, cortisol is also found to be elevated (Nolten et al., 1980; Weerth and Buitlaar, 2005) with blood pressure, fat deposition and impaired glucose tolerance (IGT) also elevated during gestation. Smokers often exhibit a higher degree of central obesity and elevated cortisol (Szostak-Wegierek et al., 1996). In individuals with depressive illness, cortisol activity is known to be elevated (Mortola et al., 1967). It is also frequently observed that depressive states are often accompanied by increased visceral adiposity (Weber-Hamann et al., 2002).

Animal models of obesity further support a theory of glucocorticoid excess. A number of studies have highlighted hypercortisolaemia in the Zucker rat (Cunningham et al., 1986) and a diminished response to corticotrophin-releasing factor (CRF) which can be reversed by an adrenalectomy (Freedman et al., 1986). Similar observations have been noted in the OB/OB mouse (Dubuc et al., 1986). Such studies provide useful support for the suggestion that glucocorticoid activity is associated with central obesity. These observations also suggest that the effects of glucocorticoids may be transient and that their consequences will only occur for the length of the abnormality (Rivera and Svec, 1989).

Further, support for the postulation that there is an apparent glucocorticoid excess derives from the observation that individuals with central obesity show less glucocorticoid suppression following administration of the synthetic glucocorticoid dexamethasone (DEX). This has been demonstrated in both obese women (Pasquali et al., 2002) and in high waist-hip ratio men (Ljung et al., 1996). When stimulated with

synthetic glucocorticoids, the normal physiological response of the HPA axis would be negative feedback to suppress further cortisol secretion. Reduced suppression of cortisol following DEX administration indicates a poorly regulated HPA axis. Another test to explore HPA responsiveness is to administer corticotrophin-releasing factor (CRF), which in normal individuals should prompt the HPA axis to respond by secreting cortisol. In individuals with central obesity, this response is diminished in both men and women (Katz et al., 2000; Vincennati and Pasquali, 2000).

Research has provided some support for the observed glucocorticoid excess hypothesis but there is also a wealth of research that runs contrary to this hypothesis. For example, experimental findings which show that levels of circulating plasma and salivary cortisol are not elevated in those exhibiting central obesity and are often lower than in those individuals with a peripherally obese or lean body shape. For example, Glass et al. (1981) demonstrated that circulating glucocorticoids (both basal and following DEX suppression) are normal in individuals with central obesity. Glucocorticoid hypersecretion is still a viable explanation for this. However, these alterations are not observed in baseline circulating plasma or salivary cortisol measurements. Hence, it has recently been suggested that individuals with central obesity display increased cortisol clearance capability (Lottenberg et al., 1998). In an examination of the pharmacokinetics of cortisol in central obesity, Lottenberg et al. (1998) found that high waist-hip ratio individuals displayed a greater ability to remove cortisol during glucocorticoid hypersecretion which resulted in lower measured plasma cortisol. Lottenberg et al. (1998) noted an increased level of cortisol metabolites excreted in the urine of high waist-hip ratio participants who also showed lower basal cortisol. Several studies have supported this observation. Lower morning cortisol levels (as part of the diurnal cortisol profile) have been found in both general obesity (Strain et al., 1980) and in central obesity (Ljung et al., 1996; Marin et al., 1992). It has therefore been suggested that in central obesity, cortisol turnover is much greater than normal (Strain et al., 1980) and occasionally, though not always, results in a net increase of cortisol, marked by an increase in excreted urinary metabolites. Further, Duclos et al. (1999) observed *lower* salivary cortisol concentrations across the diurnal period in comparison to lean or peripherally obese individuals, in addition to elevated urinary cortisol excretion.



Research has explored the suggestion that cortisol clearance is in some way enhanced in the centrally obese via the action of  $5\alpha$  and  $5\beta$  reductase enzymes. These enzymes are the primary catalysts for the breakdown of cortisol in the liver. Animal studies have shown that activity of these enzymes is elevated in the liver of obese rodents (Livingstone et al., 2000). In centrally obese humans, increased excretion of  $5\alpha$  and  $5\beta$  reductase metabolites has been observed (Andrew et al., 1998; Fraser et al., 1999; Reynolds et al., 2001). The increased inactivation of cortisol by these enzymes may explain why observed basal cortisol is normal or low in central obesity when compared to peripheral or non-obese individuals (Ljung et al., 1996; Walker et al., 2000). It is therefore, possible that the HPA axis over-secretes as a compensatory mechanism and this results in the accumulation of visceral fat in central obesity (Bjorntorp, 1991). However, the proposed mechanism of action of these catalysing enzymes is still not clear. Westerbacka et al. (2003) found that  $5\beta$  reductase was associated with an increase in body fat but not specifically, central fat accumulation.

If it is the case that glucocorticoid hypersecretion occurs in the centrally obese but that the rate of removal is elevated to such a degree that it is difficult to detect in basal secretion, to what degree would individuals need to be exposed to glucocorticoid excess in order for negative health effects to become apparent? On a basal level cortisol/glucocorticoid secretion rates appear normal but in response to a stressor, cortisol responses are significantly elevated in the centrally obese compared to peripherally obese or lean individuals. Whether frequent stress exposure is sufficient for the manifestation of poor health remains to be determined but it seems plausible on the basis of the suggestive evidence outlined above.

The suggestion that elevated cortisol occurs as a result of chronic exposure to stress (particularly psychological stress), which increases vulnerability to stress induced cortisol secretion and results in fat deposition (Bjorntorp, 1991; Rebuffe-Scrive, 1991) is well documented and will be discussed further in Section 1.5.3.3.

### **1.5.3.2 The Cortisol Awakening Response & Cortisol Profile in the Centrally Obese**

Although it is reasonably well accepted that basal cortisol in the centrally obese is frequently normal or slightly lowered, compared to controls, the 24-hour circadian

rhythm of cortisol and the cortisol awakening response in the centrally obese has yet to be examined. It is interesting to note that some earlier studies examined basal cortisol in the obese during the early morning and found cortisol to be lowered compared to non-obese individuals (e.g. Simkin et al., 1961; Szenas and Pattee, 1959). However, this research was conducted prior to the identification of the cortisol awakening response. The dexamethasone (DEX) suppression test as previously discussed in Section 1.5.3.1, has offered some insight into the basal activity of cortisol in the centrally obese. Females with central obesity demonstrate poor cortisol suppression post DEX administration (Pasquali et al., 2002) and as do males with high waist-hip ratios (Ljung et al., 1996). In terms of cortisol profiling, those who demonstrate a clear cortisol response to waking and low evening cortisol showed effective DEX suppression (for example lean individuals). Those with flattened or blunted cortisol awakening response profiles showed elevated cortisol post DEX administration. The combination of a flattened cortisol profile and poor DEX suppression are arguably characteristic of repeated or chronic challenges to the HPA axis (Dallman, 1993; Chrousos and Gold, 1992) and are interestingly common in individuals with central obesity (Ljung et al., 1996; Marin et al., 1992).

It has been presumed that basal cortisol is altered as a consequence of chronic over-activation of glucocorticoid receptors as discussed in Section 1.1.2. The circadian profile demonstrates periods of very low cortisol activity and very high activity requiring effective HPA regulation at both ends of the scale to maintain a stable basal diurnal profile. Studies in rats (Spencer et al., 1993) have demonstrated a circadian trough in corticosterone (cortisol in humans) activity, which is reflected in the human circadian profile. Low levels of cortisone/cortisol secretion activate only MR receptors and fail to activate GR receptors (Spencer et al., 1993). When cortisone/cortisol level is raised from a trough, the HPA axis compensates by lowering peak levels at waking (Akana et al., 1992) such that the net cortisol secretion during the 24-hour period does not change. In humans, elevated nocturnal cortisol is characteristic of chronic stress as well as depression, mania, ageing and fasting (Cella et al., 1995; Linkowski et al., 1985; Linkowski et al., 1987; Van Cauter et al., 1996). Nocturnal cortisol elevation results in a compensatory normalisation or reduction in morning cortisol activity (peak levels) which can lead to subtle but insignificant rise in daily mean cortisol output (Van Cauter et al., 1996). This rise in night time activation is sufficient to induce GR receptor

activity and increase vulnerability to the negative effects of stress exposure and central obesity.

Some studies have demonstrated a negative relationship between waist-hip ratio and morning cortisol level (measured between 0800 and 0900 hours) (Andrew et al., 1998; Ljung et al., 2000; Phillips et al., 2000) while others have found no effect of central obesity on the cortisol awakening response (Phillips et al., 1998; Rask et al., 2002; Ward et al., 2003). Wallerius et al. (2004) reported a positive correlation between waist-hip ratio and the cortisol awakening response which suggests central adiposity and chronic stress exposure influence basal morning cortisol activity. The cortisol response to waking was also found to be positively correlated with BMI and fasting plasma glucose, insulin and triglyceride levels. Steptoe et al. (2004) explored the cortisol response to waking in a larger study of 89 males and 83 females. The findings confirmed previous observations from Wallerius et al. (2004) in that cortisol responses were positively associated with central adiposity in males (using waist-hip ratio). However, in both studies, only one day was sampled to determine cortisol activity and this may not reflect typical individual cortisol activity.

#### **1.5.3.3 Stress Exposure & Cortisol Activity in the Centrally Obese**

Stress exposure is a major contributor to the relationship between cortisol and obesity. Research suggests that chronic exposure to stress disrupts HPA axis regulation resulting in an over-secretion of glucocorticoids. This suggests that the response to stress could be elevated in individuals with central obesity when compared to peripherally obese or lean individuals. Previous research appears to support this. For example, Marin et al. (1992) found an elevated serum cortisol response in a group of centrally obese post-menopausal women compared with lean or peripherally obese individuals in response to a series of stressor tasks including the cold pressor test, colour-word (stroop), and mathematical tests. Marin et al. (1992) concluded that an increased sensitivity of the HPA axis in centrally obese females actively fuelled abdominal fat deposition. Similar results were found by Moyer et al. (1994) in a sample of centrally obese females. However, both studies failed to exclude participants with confounding characteristics for example, smokers. Smoking is known to affect both cortisol activity and the incidence of central obesity (Szostak-Wegierek et al., 1996).

Epel et al. (2000) compared both lean and overweight females on the basis of their peripheral and central fat. This comparison avoided confusing central obesity with whole body obesity, an error frequently made in previous research and a possible explanation for the lack of glucocorticoid excess observed in previous studies. Epel et al. (2000) observed a more pronounced cortisol response to a series of stressors in those individuals with central obesity. Furthermore, these results showed that high waist-hip ratio lean females (who exhibited central obesity but with a normal BMI) failed to habituate to a repeated stressor. An elevated cortisol response was observed on the first exposure to stress and also on subsequent occasions despite increased familiarity and predictability of the stress exposure. Individuals with a high waist-hip ratio, in addition to being overweight (though not centrally obese), did demonstrate habituation. Cortisol responses were elevated following the first exposure to the stressor but were followed by adaptation to the stressors on subsequent exposure. These findings concur with the research described above but can be viewed with greater confidence due to better controls. Nevertheless, the criteria used by Epel et al. (2000) for the diagnosis of central obesity (WHR calculation) differed from previous studies and from the WHO definition (WHO, 2000). Epel considered a ratio of 0.79 or greater to be high, which was considerably lower than the WHO criteria (0.85 or greater). How this affected the outcome of the study is unclear. Another limitation of the study is that only female volunteers were included and a strict set of exclusion criteria were applied. The use of very stringent exclusion criteria has been highlighted as potentially responsible for the exclusion of a key population. Obesity is associated with poorer health, insulin resistance, diabetes, hypertension, particularly in central obesity. Research tends to exclude on the basis of these conditions and symptoms. Rivera and Svec (1989) argue that as a consequence this key population is likely to be excluded and that this permits skew in data towards 'lower body proportions' or peripheral obesity, individuals who will be marked by an absence of glucocorticoid excess and fewer health problems.

High waist-hip ratio individuals may be more likely to succumb to illness more often, experience ulcers, stomach bleeding and health complaints which could be related to stress and coping strategies (Bjorntorp, 1995). It has been suggested that glucocorticoid excess arises out of chronic exposure to stress with a consequent accumulation of visceral fat. Exposure to high levels of stress results in repeated activation of the HPA axis which leads to excessive secretion of cortisol. Studies suggest an association

between high waist-hip ratio, poor coping, high stress and antidepressant use (Bjorntorp, 1995; Lapidus et al., 1989; Larsson et al., 1989). High waist-hip ratio individuals also tend to use more stimulants, smoke and consume more alcohol. This could promote the continued stimulation of the HPA axis contributing to the development of central obesity (Lapidus et al., 1989; Larsson et al., 1989). Indeed, chronic exposure to stress is a plausible explanation for the suggested glucocorticoid excess in central obesity. In Cannon's (1928) 'fight or flight' hypothesis of a classic stress response, one of the peripheral actions of the HPA axis is the suppression of sex hormones and inhibition of growth hormone from the pituitary. Previous research has suggested that sex hormones (oestrogen) are protective against the development of obesity (Vamvakopoulos and Chrousos, 1993). Chronic stress thus leads to chronic over-stimulation of the HPA axis and constant inhibition of sex and growth hormones (Laatikainen, 1991). These effects also account for the increase in obesity with age, when sex hormones and growth hormone diminish whereas cortisol and insulin, which also promotes lipid accumulation, do not (Bjorntorp, 1995). Studies have shown that administering testosterone to males with low endogenous levels and high visceral fat produces a reduction in adiposity. Similarly, administration of growth hormone to patients with growth hormone deficiency reduced visceral adiposity by 30% (Bengtsson et al., 1993). Furthermore, elevated cortisol combined with reduced sex hormone levels is often associated with an increased risk of insulin resistance (Bjorntorp, 1995).

Exposure to chronic stress provides a route to cortisol hypersecretion through HPA dysregulation. Both over- and under- feeding can result in cortisol hypersecretion (Abell et al., 1987; Galvao-Teles et al., 1976; O'Connell et al., 1973). Regulation of weight about a set point is important, and is an allostatic process driven by HPA axis activity (McEwen, 1998). However, patients with anorexia nervosa despite apparent cortisol hypersecretion do not exhibit central obesity. This may suggest that the relationship between cortisol activity and central obesity may be open to other mediating factors and is not solely based on glucocorticoid activity from overexposure to chronic stress. This suggests that other metabolic factors may be involved (Invitti et al., 1999).

In addition to the role of allostasis in feeding and weight regulation, McEwen (1998) suggests that allostatic load has a role in the basic regulation of the HPA axis. Dysregulation may be the result of neuronal damage in the suprahypothalamic region of

the brain, which is responsible for regulating the negative feedback process. This can occur as a result of early life stress or chronic exposure to stress in later life and is a plausible explanation for apparent HPA dysregulation in those with central obesity and/or metabolic syndrome symptomology. Following this suggestion, it could be hypothesised that HPA dysregulation precedes central obesity and central obesity in turn is a consequence of stress exposure.

#### **1.5.4 Stress & the Metabolic Syndrome**

The connection of the metabolic syndrome to stress has led to the implication of cortisol in the expression of the syndrome. One of the main symptoms of metabolic syndrome is central obesity, and as described in Section 1.5.3, central obesity has been clearly linked to altered cortisol secretion. The most important symptom of metabolic syndrome, insulin resistance, is also linked to cortisol activity. Cortisol, via its role in gluconeogenesis is involved in the expression of impaired glucose tolerance and insulin resistance in the centrally obese (See Section 1.2.1). Stress is also implicated in inflammatory responses. Stress activates an acute phase response (APR) which is an innate immune response resulting in the release of acute phase proteins and this process is associated with the onset of metabolic diseases (Black, 2003).

The proposed role of stress in the manifestation of the metabolic syndrome led to the suggestion that cortisol directly underlies the expression of syndrome and that metabolic syndrome is a neuroendocrine disorder (Bjorntorp and Rosmond, 2001). Rosmond et al. (1998; 1999b) assessed salivary cortisol over one working day in relation to central obesity. They observed that pathological cortisol secretion occurred in a small number of males who also demonstrated symptoms of the metabolic syndrome. Rosmond et al. (1998; 1999b) argue that altered cortisol secretion and HPA dysregulation are important contributors to the manifestation of related metabolic syndrome symptomology.

Cross-cultural studies have also shown that increased glucocorticoid action may explain ethnic differences in the prevalence of metabolic syndrome and more interestingly from the point of view of the research conducted in this thesis, in those women with a higher body mass index (Ward et al., 2003). Research has also considered the possibility that abnormal HPA regulation may be the result of genetic alterations at the glucocorticoid receptor gene locus that predispose to the development of central obesity (Rosmond et

al., 2000). Further support for the association between cortisol and metabolic syndrome comes from the observation that altered cortisol secretion has often been associated with insulin resistance and abnormalities in lipid metabolism including elevations in blood pressure and hypertension (Rosmond and Bjorntorp, 2001).

Taken together, the evidence suggests that altered cortisol secretion is an important factor in the development of metabolic and cardiovascular risk factors and ultimately, the development of a metabolic syndrome. The metabolic syndrome *is* currently viewed as a neuroendocrine disorder with research increasingly exploring glucocorticoid activity in relation to observed metabolic symptomology (Bjorntorp and Rosmond, 2000; Gale et al., 2002; Khani and Tayek, 2001; Rosmond and Bjorntorp, 2001). However, it is important to note that research conducted since Bjorntorp and Rosmond, (2000) initial proposal, segregates in terms of those who find cortisol alterations in association with metabolic syndrome symptoms and those who do not. For example, Ward et al. (2004) assessed basal salivary cortisol responses in a group of older adult males and found no association between level of cortisol output and metabolic syndrome symptomology. Further to this, Kajantie et al. (2004) explored the relationship between cortisol and birth weight and failed to find any associations between cortisol and metabolic syndrome and between cortisol and birth weight.

### **1.5.5 Additional Factors Influencing Cortisol in the Centrally Obese**

#### **i. 11 $\beta$ -Hydroxysteroid Dehydrogenase (11 $\beta$ -HSD)**

A number of metabolic factors have been considered in an attempt to explain the apparent glucocorticoid excess in central obesity. One important factor is the activity of the enzyme 11 $\beta$ -Hydroxysteroid Dehydrogenase (11 $\beta$ -HSD). 11 $\beta$ -HSD is an enzyme that acts on cortisol to assist the conversion of inactive cortisone to active cortisol. 11 $\beta$ -HSD has been shown to regulate corticosteroid action at the pre-receptor level and exists in two isoforms (Albiston et al., 1994; Tannin et al., 1991). Low affinity 11 $\beta$ -HSD1 controls the conversion of cortisol to cortisone and vice versa and is predominantly expressed in hepatic, gonadal and central nervous system tissues where it is known to modulate glucocorticoid action (Jamieson et al., 1995; Whorwood et al., 1995). 11 $\beta$ -HSD2 is the high affinity isoform that has been shown to protect from cortisol excess, inactivating active cortisol to cortisone (Stewart, 1996). Previous research has observed that only the type 1 isoform was found in the adipose tissue of the

omentum (the collection of fat around the stomach and colon covering most of the intestine which serves to connect the viscera and support blood vessels) which could lead to the continuous generation of active cortisol from inactive cortisone (Bujalska et al., 1997). The expression of this enzyme was also increased following exposure of the omental adipose stromal cells to cortisol and insulin. This suggests continuous exposure to glucocorticoids (from repeated stress and other factors) or adipose tissue could contribute to the development of central obesity and increase the risk of developing associated health problems such as insulin resistance, glucose intolerance, and hypertension. The  $11\beta$ -HSD2 isoform has not yet been detected in the same tissue. Further exposure to cortisol and insulin has been found to produce greater expression of  $11\beta$ -HSD1 suggesting a possible 'fast-forward' mechanism of action (Bujalska et al., 1997). It is possible that even though cortisol levels remain normal, cortisol has the ability to act as a potent mineralcorticoid which would have negative health consequences (Bujalska et al., 1997). A similar pattern of activity was noted in relation to the effect of the type 2 isoform in the kidney on the production of a hypertensive state (Edwards et al., 1985). Bujalska et al. (1997) comment on the possibility that the same may occur in adipose tissue, attributable to the  $11\beta$ -HSD1 isoform and leading to the development of insulin resistance, hypertension, and dyslipidemia. However, Westerbacka et al. (2003) failed to establish a link between the activity of  $11\beta$ -HSD1 and central obesity, but was able to link the type 1 isoform and whole body obesity. Westerbacka et al. (2003) argue that the mechanisms underlying these associations remain to be fully elucidated and in light of previous research suggest that waist-hip ratio may not be an accurate enough measure for the diagnosis of central obesity.

## **ii. Catecholamines**

HPA dysregulation has been linked to the presence of abdominal catecholamines (Pasquali and Vincennati, 2000). Catecholamines modulate the activity of corticotrophin releasing factor (CRF) and adrenocorticotrophin (ACTH) secretion during acute and chronic exposure to stress. These effects are mediated by  $\alpha 1$  and  $\alpha 2$  adrenoceptor subtypes. The  $\alpha 2$  receptor subtype is known to inhibit ACTH activation and in essence is presumed to be a contributing factor to the negative feedback mechanism of the HPA axis to prevent cortisol/glucocorticoid hypersecretion. Obese women are known to show an increased ACTH response to stress (Pasquali et al., 2000)



indicating the possibility that centrally obese women may lack normal control of the  $\alpha_2$  adrenoreceptor.

### **iii. Food consumption**

Food consumption has been shown to influence cortisol secretion (Abell et al., 1987; Galvao-Teles et al., 1976; O'Connell et al., 1973). Conversely, glucocorticoids have been found to directly stimulate food intake through stimulation of neuropeptide Y (a peptide neurotransmitter). NPY stimulates feeding, insulin and interacts with glucocorticoids. NPY also reduces the breakdown of triglycerides and lowers body temperature). NPY also stimulates food intake through inhibition of corticotrophin releasing hormone (Henrichs et al., 1993; Tataranni et al., 1996; Tempel and Leibovitz, 1994). Glucocorticoids bind to glucocorticoid type 1 receptors (mineralocorticoid receptors) in the hypothalamus, which are sensitive to low concentrations of glucocorticoids and also bind to type 2 receptors when in higher concentrations (glucocorticoid receptors). It has been shown that glucocorticoid binding to these receptors is followed by increased food consumption with preferential selection of fatty foods (type 1 receptor) and carbohydrates (type 2 receptors) (Levine and Billington, 1997). It is possible that chronic stress exposure could contribute to the development of obesity by enhancing food intake via activation of glucocorticoid receptors.

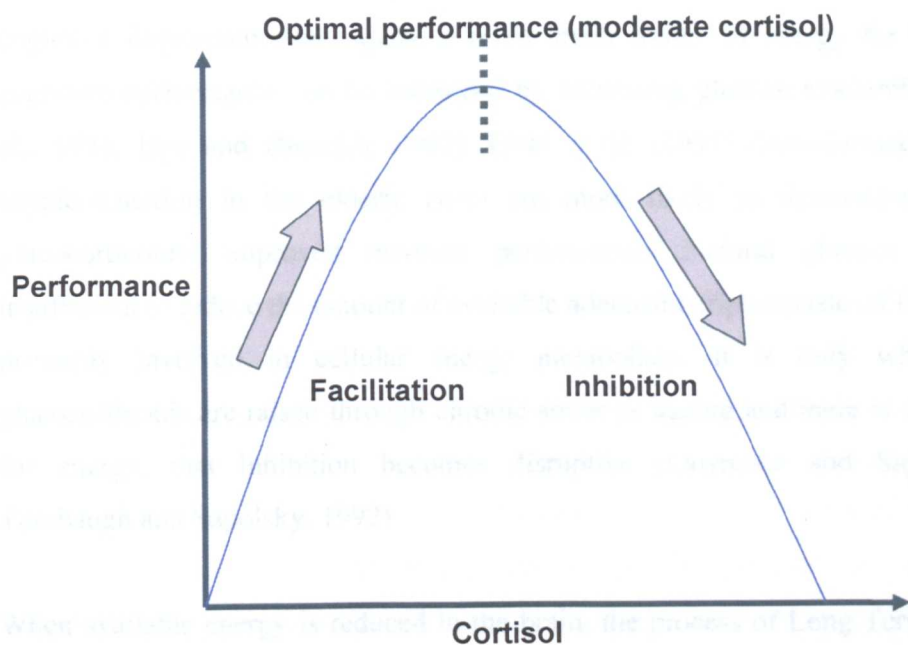
In summary, the basal cortisol diurnal profile may be different in those with central obesity reflecting a dysregulated HPA axis. Further, cortisol responses to a psychological stressor are elevated compared with lean or peripherally obese individuals. Finally, cortisol activity may be related to the biological parameters that are associated with the metabolic syndrome.

## **1.6 Cortisol & Cognitive Performance**

The literature exploring the impact of glucocorticoids on cognitive performance is extensive, dating back to the 1950's when health professionals used corticosteroids as a form of treatment for rheumatoid arthritis and asthma. It was discovered that corticosteroid treatment had significant side effects on mood and cognition labelled as 'steroid psychosis' (Clark et al., 1952). Subsequent research has examined many aspects of glucocorticoid activity in relation to cognition and is discussed below.

### 1.6.1 Mechanisms of Action – MR & GR Receptors & the Hippocampus

The hippocampus is an important area of the brain for learning and memory with a key role in declarative memory, spatial memory and the processing of emotional stimuli (De Kloet et al., 1998; McEwen et al., 1995; McEwen, 1997). The hippocampus contains a large number of MR and GR receptors reflecting a high level of glucocorticoid activity (McEwen et al., 1986) as discussed in Section 1.1.2. The dynamics of the activation of these receptors modulates the effect of cortisol on cognitive performance. The combined activation of the receptors produces a dual effect on cognition reminiscent of the Yerkes-Dodson inverted 'U' hypothesis (Figure 1.3) for the effect of arousal on performance (Luine et al., 1993).



**Figure 1.3:** Adaptation of the Inverted 'U' Hypothesis of Stress-Induced Cognitive Impairment

High occupation of the MR receptors in conjunction with high occupation of the GR receptors would produce deficits in cognitive performance. Similarly, at the lower end of the spectrum, too little activation of the MR and GR receptors should also result in cognitive deficit. An MR/GR receptor balance is required to ensure optimum cognitive performance. Over-activation of MR and GR receptors in the hippocampus leads to stress induced memory impairments specifically in declarative memory. Increased activity of the receptors within the hippocampus due to chronic stress, ageing, or glucocorticoid exposure causes a down regulation of the glucocorticoid receptors. This is known as the 'glucocorticoid cascade hypothesis' (Sapolsky et al., 1986) in which the

role of the hippocampus in the human physiological stress response is emphasised. Down-regulation of MR/GR receptors in the hippocampus can interfere with the negative feedback system of the HPA axis. This causes secretion of excess levels of glucocorticoids and leads to a down-regulation of the receptors in the hippocampus. This can lead to neuro-degeneration and hippocampal atrophy (Starkman et al., 1992).

In addition to a direct impact of glucocorticoids on the hippocampus, neuronal loss can also occur from a disruption of neuronal energy metabolism. Glucocorticoids have been shown to prevent glucose transport into the brain in humans (De Leon et al., 1997), in cultured hippocampal neurons and glia (Horner et al., 1990; Virgin et al., 1991). A reduction in glucose availability as a result of this inhibition could explain observed cognitive impairment since glucose is the main source of energy for the brain and cognitive performance can be enhanced by increasing glucose availability (Benton et al., 1994; Dye and Blundell, 2002). Gold et al. (1987) demonstrated that glucose supplementation in the elderly (who are more likely to demonstrate high basal glucocorticoids) improved memory performance. Natural glucose inhibition is insufficient to reduce the amount of available adenosine triphosphate (ATP), a molecule primarily involved in cellular energy metabolism. It is only when levels of glucocorticoids are raised through chronic stress or ageing and there is a high demand for energy, that inhibition becomes disruptive (Lawrence and Sapolsky, 1994; Tombaugh and Sapolsky, 1992).

When available energy is reduced in the brain, the process of Long Term Potentiation (LTP) is affected. Long-term potentiation facilitates the consolidation and storage of new memories a process that has been shown to rely on NMDA receptors in the cornu ammonis region one (CA1) of the hippocampus (for a review see Alderson and Novak, 2002). Selective activation of the glucocorticoid receptors in the hippocampus has been shown to increase the rate of LTP (Pavlides et al., 1994; Pavlides et al., 1995) but high levels of glucocorticoids suppress LTP (Pavlides et al., 1993; Pavlides et al., 1995) and have even been shown to encourage Long Term Depotentiation (LTD) (Pavlides et al., 1995). Similarly, glucocorticoids have demonstrated an effect on the excitability of hippocampal neurons interfering with the electrophysiology of the hippocampus. Exposure to elevated glucocorticoids results in reduced excitability (Joels and De Kloet, 1992; Zeise et al., 1992). This action is again biphasic and corresponds to an inverted

'U' hypothesis (Luine et al., 1993) and altered LTP (Pavlidis et al., 1994; Pavlidis et al., 1995). In conjunction with proposed glucose inhibition, these findings could account for a collective modulatory effect of glucocorticoids on cognitive performance.

### **1.6.2 Effects of Endogenous Cortisol on Memory**

The hippocampus is an important component of the relationship between glucocorticoid activity and cognition as previously discussed. It is the region of the brain frequently associated with memory, its encoding, consolidation and retrieval particularly in declarative and spatial memory (De Kloet et al., 1998; McEwen et al., 1995; 1997). In general, glucocorticoids have been shown to enhance memory consolidation but to impair retrieval and aspects of working memory as will be discussed. The impact of glucocorticoids on performance can be explored in relation to acute changes in glucocorticoid, for example, due to external stressors in both young and older adult populations and will be discussed in the next section.

The most common method of studying the impact of acute stress induced glucocorticoids on cognitive performance is by first exposing the individual to some form of external stressor and measuring subsequent performance. Many studies follow this procedure; the majority have found that acute exposure to stress is sufficient to produce cognitive impairment. Lupien et al. (1997) exposed a sample of healthy elderly subjects to a public speaking stressor task (a variation on the TSST) and discovered that subsequent declarative memory performance was impaired in those who demonstrated a cortisol response to the stressor. No effects were reported for non-declarative memory. Further, the results demonstrated that those who responded to the stressor also demonstrated an anticipatory rise in cortisol 60 minutes prior to stress exposure. This in conjunction with actual stress responses as a result of stress exposure could have influenced performance and was sufficient to produce impairment (Lupien et al., 1997). However, it is possible that this anticipatory rise may have been due to pre-experimental baseline cognitive testing administered prior to stress exposure, which could itself have been a stressor. For example, Bohnen et al. (1990) used a cognitive test battery as a stress induction tool, in a sample of middle-aged subjects. However, the results showed that those who exhibited the greatest cortisol rise (responders) were impaired in attentional processing. Domes et al. (2002) exposed a sample of healthy post-menopausal females to the Trier Social Stress Test and found no impairment in memory

performance. Post hoc analysis of the data revealed that those exhibiting an elevated cortisol response to the stressor demonstrated better performance than those who did not respond. It is possible, however, that the stress induction was insufficient to raise cortisol levels to a point of impairment. Further, de Quervain et al. (2000) proposed that it is not possible to determine which aspect of memory is impaired since exposure to the stressor occurred prior to learning. Should exposure occur after learning and during retrieval, then impairment will be observed (de Quervain et al., 2000). This further supports the theory that an MR/GR receptor balance is vital for optimum performance, from the observation impairment was observed in those demonstrated a cortisol elevation post stressor, hence shifting the receptor balance.

Wright et al. (2005) failed to find an association between basal cortisol activity and subsequent memory performance but did find that cortisol responsivity was inversely related to cognitive performance. The findings demonstrated that a better recovery of heart rate (in both males and females) and blood pressure (in males) post stress exposure was associated with superior memory performance.

These findings suggest that acute stress exposure can be sufficient to cause cognitive impairment. Discrepancies in the findings are often due to the implementation of different types of stressors and also the use of different methods of cognitive testing. These may account for the observed differences in performance and should be considered when evaluating research findings.

### **1.6.3 Effects of Exogenous Cortisol on Memory**

An alternative method of assessing the influence of glucocorticoids on cognition is by exogenous pharmacological intervention. This may be more likely to produce a cortisol elevation whereas some stress induction tools may be ineffective. This method also allows for easy control of timing and dosage. A dose-response study was conducted by Beckwith et al. (1986) administering hydrocortisone. Doses of 5, 10, 20 and 40mg were administered and short and long-term memory assessed 60 minutes post administration. Glucocorticoid administration at all dosage levels facilitated recall during the first few presentations but only the higher doses continued to enhance recall when task load (number of words to recall) was increased. The hydrocortisone, however, was administered in a glucose drink. Glucose alone is known to enhance memory

performance (e.g. Benton et al., 1994) and so the resultant impact on memory is unlikely to be solely related to glucocorticoid administration. In terms of cognitive impairment, Kirschbaum et al. (1996) found that administering 10mg of hydrocortisone significantly reduced performance specifically in delayed recall (declarative memory) 60 minutes post administration. Similarly using prednisone, Wolkowitz et al. (1990; 1993), found that high doses (80mg) were sufficient to disrupt long term recall (over one week). The number of errors made increased in comparison to a daily low dose of DEX (1mg daily) which suppresses glucocorticoid activity and which failed to have an effect on memory. However, Newcomer et al. (1994) did find impairment in declarative memory (acquisition and recall) from suppressed glucocorticoid activity after four days of exogenous DEX administration (doses of 0.5, 1, 1, and 1mg per day). No impairments were noted for non-declarative memory or attentional processing. The findings previously discussed (Newcomer et al., 1994; Wolkowitz et al., 1990; 1993) were attributed to the inverted 'U' hypothesis for the modulatory effects of glucocorticoids on cognitive performance with each study highlighting impairment from too little or too much glucocorticoid exposure.

In a double-blind study, Schmidt et al. (1999) found that high doses of prednisone in young males impaired object recall up to four days post administration. Similarly, Young et al. (1999) found impaired paired associates learning performance (a test of declarative memory) following hydrocortisone administration over a ten-day period (20mg twice daily). Impairments in both spatial and working memory tasks were also observed to confirm earlier observations in endogenously administered glucocorticoids (e.g. Lupien et al., 1999b). Indeed, Young et al. (1999) observed deficits in spatial and working memory and suggested that glucocorticoids could also mediate a frontal lobe dysfunction in addition to hippocampal-related processing. It would be of importance to make a global assessment of cognitive performance to determine in which cognitive domain these deficits occur.

#### **- Influence of Time of Day**

The time of day when exogenous administration occurs is of importance. Endogenous glucocorticoids follow a circadian rhythm as previously described (Section 1.3); therefore there will be a differential activation of the MR/GR receptors at in the AM phase than in the PM phase (DeKloet et al., 1999). Consequently, the relationship

between glucocorticoids and memory may be a function of time of day (Lupien et al., 2002a; Maheu et al., 2005). Fehm-Wolfsdorf et al. (1993) administered 50mg oral hydrocortisone to a group of young healthy subjects in the AM phase followed by a free recall task. Elevating cortisol further during the morning period, when cortisol is naturally highest, disrupted performance. The same level of administration had no effect in the evening. These findings are consistent with studies of the influence of endogenous glucocorticoid on cognition as discussed previously.

#### **- Influence of Dosage Timing**

Pharmacological studies add further support to the emerging view that the time of glucocorticoid elevation is important in terms of determining specific effects on cognitive performance. Lupien et al. (1995) compared fixed doses of hydrocortisone (40µg/kg, 300µg/kg, and 600µg/kg) with comparable doses of saline. The results showed a dramatic decrease in memory performance in those who had shown the greatest increase in cortisol post drug administration and when learning had occurred during the infusion period. The same pattern of results was not evident for those who undertook learning after the infusion or those who had not demonstrated a significant response to hydrocortisone administration (Lupien et al., 1995). de Quervain et al. (2000) suggested that the timing of the glucocorticoid elevation is important, with glucocorticoids administered during learning subsequently weakening memory consolidation. This was further confirmed by Lupien et al. (1995). de Quervain et al. (2000) observed that glucocorticoid elevations (exogenously produced) one hour prior to learning failed to affect memory retrieval. de Quervain et al. (2000) permits that glucocorticoids will only affect recall of information when the cortisol elevation occurs during the actual retrieval process itself. This suggests that glucocorticoid elevations differentially affect encoding, storage and retrieval (de Quervain et al., 1998).

#### **- Influence of Age**

Consistent with studies of endogenous administration of glucocorticoids, differential effects are noted between younger adults and older adults/elderly populations. Newcomer et al. (1995) found that administration of higher DEX treatment (1, 2, 3 and 4mg per day) resulted in better immediate and delayed declarative memory performance in a younger population but showed no effects in an elderly population. Lupien et al. (1994) argued that as an elderly population exhibit generally higher basal levels of

glucocorticoids, administration of DEX could be beneficial if it reduces glucocorticoid activation and this would explain the absence of change in memory performance. Porter et al. (2002) administered hydrocortisone to an elderly population and failed to find any effect on memory performance despite cortisol levels at testing being ten fold compared to a placebo condition. It is possible that the elderly individuals in this study exhibited high basal cortisol and lacked sensitivity to changes in glucocorticoid concentration due to disruption in the MR/GR balance resulting from chronic glucocorticoid exposure. This supports Sapolsky et al.'s (1986) glucocorticoid cascade hypothesis in that it reflects down-regulation of glucocorticoid receptors (Sapolsky et al., 1986).

In a double blind study, Lupien et al. (1995) lowered basal glucocorticoid levels through administration of metyrapone (a glucocorticoid synthesis inhibitor) before restoring glucocorticoid levels by administration of hydrocortisone. In those with high basal cortisol, a chemical lowering of glucocorticoids had no effect on memory performance. Restoring glucocorticoids to baseline using hydrocortisone resulted in significantly impaired memory. In contrast, those with moderate basal cortisol demonstrated a significant impairment of memory when glucocorticoid reduction was taking place. Administration of hydrocortisone restored glucocorticoid levels and hence restored performance. Lupien et al. (2002a) suggested that the observed impairment in those with high basal cortisol was the result of diminished numbers of MR receptors and consequent heightened sensitivity to GR receptors. The lowering of glucocorticoid levels had no effect due to the lower number of MR's available for binding and greater binding to the GR receptor. The glucocorticoid replacement produced impairment due to faster saturation of the GR receptors because of the reduced number of MR receptors. For example, in a longitudinal study, Wetzel et al. (1995) demonstrated a reduced number of MR's as result of excessive glucocorticoid exposure in aged subjects who showed consistently increasing basal cortisol levels over time with current high basal levels. Further, Lupien et al. (2002) demonstrated that chemically lowering glucocorticoids (using metyrapone) impairs performance in younger adults who exhibit lower basal cortisol levels. Administration of hydrocortisone restored glucocorticoid levels and as a result, restored performance. Hence, lowering cortisol in individuals who already exhibit low basal glucocorticoid levels, results in poorer cognitive performance. This contributes to the growing body of evidence that supports the changing MR/GR balance across the lifespan. However, De Kloet et al. (1999) argue that exogenous



glucocorticoid administration studies may be less reliable than endogenous studies because context is important in determining steroid mediated effects. Moreover, synthetically administering glucocorticoids does not allow for differential receptor activation and are not administered in what could be described as a natural order of environmental input during information processing (De Kloet et al., 1999). The deficits observed are not natural responses but rather ‘opportune’ responses to an ‘out of context’ steroid simulation. Nevertheless, studies using exogenous glucocorticoid administration mirror the results studies which used stress induction tools and studies of endogenously raised glucocorticoids.

#### **1.6.4 Influence of Emotion**

Research has suggested that the influence of stress induced glucocorticoids (cortisol) on memory depends on emotional state (Abercrombie et al., 2006; Elzinga and Roelofs, 2005). This is postulated to be linked to the influence of the limbic system on the HPA axis, in particular the basolateral nucleus of the amygdala, crucial for the processing of emotional material (Anderson and Phelps, 2001). The effect of cortisol on noradrenergic processing within the amygdala is necessary for its effects of memory (reviewed by Roozendaal, 2000; Van Stegeren et al., 2004). Glucocorticoids have shown no effect on memory for emotional material when there is no activation of the basolateral nucleus of the amygdala (Roozendaal and McGaugh, 1997; Roozendaal et al., 1999). Elevations in glucocorticoids have been shown to enhance the consolidation of emotional material. For example, Buchanan and Lovallo (2001) observed a facilitation of memory for emotional material following an exogenous cortisol administration (20mg). Further, Elzinga et al. (2005) found a stronger recall for emotional material compared to neutral material. Kuhlmann et al. (2005) observed specific impairment in the retrieval of negative words compared with neutral words in a 5hour delayed recall task. This confirms the negative effect of glucocorticoids on memory retrieval but support the hypothesis that emotional material is more sensitive to the modulatory effects of glucocorticoids on memory.

#### **1.6.5 Gender Differences in the Cortisol – Cognition Relationship**

A number of studies have highlighted that some of the effects of glucocorticoid levels on cognitive performance are gender specific. Kalmijin et al. (1998) reported a non-significant trend for males to experience longitudinal increases in free cortisol compared

to females yet Lupien et al. (1995) failed to find any gender differences in a study of a similar duration. Animal studies have reported differential effects in male and female rats with stress enhancing classical conditioning in males more than in females in whom it is impaired (Woods and Shors, 1998). Similarly in humans, stress has been shown to impair declarative memory in females compared to males (Seeman et al., 1997; Wolf et al., 1999) affecting declarative memory performance. Females who showed impaired declarative memory had higher basal glucocorticoids.

The prevalence of some psychiatric conditions such as depression which may involve a dysregulated HPA axis is 2x greater in females (Breslau et al., 1997; Desai and Jann, 2000). However, confounding variables that are often difficult to control for may be more common in women. Many studies fail to consider menstrual phase, menopausal status, or oral contraceptive use (for example in de Quervain et al. 2000 and Newcomer et al., 1999). These may have had an effect on cognitive performance, leading to observed heightened responsivity and poorer performance in females that was inflated. Wolf et al. (2001) explored potential gender differences and found that males demonstrated a more pronounced association between stress-induced glucocorticoids and performance on a verbal recall task than did females. The observation that females were seemingly protected from the effect of the stressor was attributed to the protective effects of oestrogen against stress (Galea et al., 1997) and the possibility that estradiol modulates the relationship between cortisol and memory (Carlson and Sherwin, 1999).

#### **1.6.6 Dehydroepiandrosterone (DHEA), Cortisol & Cognition**

The role of cortisol in cognition is associated with another major secretory product of the adrenal glands, Dehydroepiandrosterone (DHEA) and its sulphate ester DHEAS. DHEA shows a significant decrease with age whereas it is presumed that cortisol levels can remain stable or may show an increase (Laughlin and Barrett-Connor, 2000). Research has indicated that DHEA may prevent stress-induced suppression of the immune system (Kalimi et al., 1994) but the literature which examines such associations is limited. Due to a small number of positive outcomes DHEA(S) has been labelled as a functional anti-glucocorticoid (Kalimi et al., 1994; Wright et al., 1992). However, research which substantiates these claims is minimal. Of relevance to the research presented in this thesis, DHEA has been linked to cognitive performance via its relationship with cortisol. Cortisol and DHEA are linked as a ratio of action. A low

DHEAS: high Cortisol ratio is associated with cognitive impairment, particularly in the elderly based on epidemiology studies (Kalmijin et al., 1998). DHEA demonstrates a circadian rhythm with a similar profile to cortisol that has been shown to diminish with age and appears unaffected by cortisol circadian activity (Ceresini et al., 2000). As DHEA activity exists as a ratio with cortisol, a number of studies have explored the treatment potential of DHEA in Cushing's syndrome as DHEA levels are lowered in this condition. Laureti et al. (2000) find it to be mildly successful in the short term in reducing cortisol activity. Similarly, basal DHEA levels have been found to be chronically suppressed in diabetic patients (Yamaguchi et al., 1998). Hucklebridge et al. (2005) found the mean level of DHEA over a two-day period to be positively correlated with the mean of cortisol but with greater stability when compared with daily variances in cortisol activity.

While obesity tends to increase as a result of ageing, DHEA levels decrease. In non-obese pre-menopausal women, De Pergola et al. (1991) found that DHEA and body mass index were inversely related. Yet the same was not true for obese individuals, where the relationship was less clear (De Pergola et al., 1996). Other studies have failed to find an association between obesity and DHEA (Williams et al., 1993).

#### **- DHEA & Cognition**

DHEA supplementation is postulated to improve cognitive performance and mood, but the mechanism of action is yet to be fully established. Van Niekerk et al. (2001) found no significant effects of DHEA on mood or cognitive performance following a three month DHEA treatment program in older adult males and thus little evidence for the benefits of DHEA supplementation. It is possible that DHEA has an anti-glucocorticoid effect, rather than directly enhancing cognitive performance. Wolf et al. (1997) found that following a single administration of DHEA to a younger adult population, cortisol levels immediately reduced but there was no effect on cognitive performance. Similarly, Wolf et al. (1998) found no effect of DHEA supplementation after exposure to stress in an elderly population. No effect of DHEA was noted on declarative memory however attention improved. Again, these mixed findings do not offer support for the role of DHEA as an anti-glucocorticoid nor do they provide strong evidence that it is actively involved in the modulation of cognitive processes.

### 1.6.7 Cognitive Performance in the Centrally Obese

Few studies have examined the direct impact that central obesity may have on cognitive performance. Jagust et al. (2006) found that greater waist-hip ratio was negatively associated with hippocampal volume. More specifically a one standard deviation increase in waist-hip ratio was associated with a 0.2 standard deviation decrease in hippocampal volume in addition to a 27% increase in white matter hyperintensities. Reduced hippocampal volumes and white matter hyperintensities have both been shown to significantly contribute to the development of dementia (Wu et al., 2002). The results of this study indicate that central obesity may contribute to neurodegenerative deficit.

11 $\beta$ -HSD (see Section 1.5.5i) is postulated to be a key factor in cortisol related obesity. The interaction of 11 $\beta$ -HSD and obesity on cognition is little researched but it has been observed that 11 $\beta$ -HSD1 knockout mice show improvements in cognitive performance suggesting a collective influence of metabolic factors on cognition. Further, administration of the 11 $\beta$ -HSD1 inhibitor carbonoxelone has been shown to improve memory performance in diabetics (Sandeep et al., 2004). Kilander et al. (1997) and Sorensen et al. (1982) have linked obesity with poorer cognitive performance yet neither study included females, nor was the influence of obesity on cognition the primary endpoint. It is possible that obesity is not viewed as being a direct pathway to cognitive impairment, but exacerbates pre-existing vulnerability in those with a tendency for impaired glucose regulation (Elias et al., 2005).

Cognitive decrement has been associated with impaired glucose tolerance (Hiltunen et al., 2001; Vanhanen et al., 1998) and type 2 diabetes (Bent et al., 2000; Goldstein et al., 2001; Strachan et al., 1997). A recent review by Messier et al. (2005) concluded that subtle impairments in glucoregulation can result in cognitive impairment (Convit, 2005; Craft, 2005; Ryan, 2005). Poorer glucoregulation has been associated with worse performance on tests of working memory, executive function and declarative memory (Messier et al., 2003). The observation that obesity is a cofactor in the incidence of impaired glucose tolerance, diabetes and the metabolic syndrome suggests that obesity could be linked to cognitive impairment. Elias et al. (2005) found a direct association between obesity and cognition in males but not females when other cardiovascular risk factors were controlled for with cumulative effects. Further, an association between the presence of diabetes and cognition was observed but that diabetes did not interact with

obesity. The findings may suggest that the mechanisms that link obesity and cognition are different to those which link diabetes and cognition even though key metabolic parameters are implicated in each (Elias et al., 2005; Ryan, 2003). Waldstein and Katzel (2006) assessed cognitive performance and found that individuals with a greater waist circumference were impaired on tasks of manual dexterity, motor speed, and executive function. These findings were only of significance when blood pressure was also elevated. Neither of the two aforementioned studies examined cortisol levels, nor was any observed elevation in blood pressure due to stress exposure, indicating scope for the studies presented in thesis to build on these findings.

### **1.7 Summary**

This literature review has explored the characteristics of salivary basal cortisol and cortisol in response to stress. It has also identified and explored factors which may affect cortisol response and has examined in detail the proposed relationship between cortisol and central obesity. This review highlights that the current understanding of the relationship between cortisol, central obesity and cognitive function is limited. It appears that cortisol responses are altered in central obesity and that this has implications for future health. In relative terms, research into central obesity based on waist-hip ratio is a novel area. Our understanding of the role of cortisol in the development of central obesity and in response to psychological stress is also relatively under-developed. The literature reviewed has highlighted the importance of basal cortisol, the diurnal profile and the cortisol awakening response. The factors which may affect cortisol response have been examined and are important to consider in the research presented in this thesis. Cortisol activity is associated with changes in cognitive performance in young and older adult/elderly samples. Research to date has failed to examine cortisol, central obesity and cognition concurrently. Future research to explore the link between cortisol responsivity, central obesity and cognitive function has clear implications in a society where obesity is reaching epidemic proportions. This coupled with the large body of knowledge about the health effects of obesity and metabolic syndrome, makes the research outlined in this thesis, a novel and timely avenue for investigation.

## CHAPTER TWO

### Aims and Objectives

---

---

#### 2.1 General Aims

The main aim of this thesis was to explore salivary cortisol secretions, both basal and in response to stress, in individuals who exhibit central obesity compared with those who do not. Elevated responses to psychological stress in individuals with central obesity in conjunction with obesity related metabolic parameters may influence cognitive performance.

This thesis presents a detailed exploration of stress responsivity in the centrally obese (high waist-hip ratio) compared with lean or peripherally obese individuals (low waist-hip ratio). An assessment of baseline salivary cortisol using waking synchronised saliva samples was conducted to compare the diurnal profiles of those who exhibit central obesity and those who do not. The cortisol response to stress using the psychological laboratory stressor, the Trier Social Stress Test was assessed. A novel component of this thesis explored the proposed vulnerability of the centrally obese to a cortisol mediated cognitive deficit using a validated cognitive test battery (CANTAB) to administer neuropsychological tests sensitive to hippocampal processing (declarative memory and spatial memory). The main hypothesis was that those who exhibit central obesity will show greater stress responsivity marked by an exaggerated cortisol response and poorer cognitive performance.

#### 2.2 Basal Cortisol in High/Low Waist-Hip Ratio (WHR) Individuals

##### 2.2.1 Reproducibility & Consistency of Cortisol Diurnal Profiles

Previous studies of basal diurnal cortisol have relied on volunteer sampling protocols in a free-living environment. This is often confounded by the issue of participant

compliance. There have been discrepancies in the number of days sampled in order to identify an individual's typical diurnal profile and the number of samples required to correctly display a basal diurnal cortisol profile.

A pilot study (Chapter Four) was conducted to explore the practicalities and feasibility of studying differences in basal cortisol activity using saliva sampling methods. Using a small sample of volunteers, cortisol samples were collected over a three day period at set time points synchronised to waking. The objectives of this study were to assess (i) the ability to reproduce the cortisol diurnal profile based on salivary cortisol samples (ii) the consistency of the cortisol diurnal profile across the three day period and (iii) to explore participant compliance with a strict salivary cortisol sampling protocol under free-living conditions. The study aimed to establish the reproducibility and consistency of the cortisol awakening response and diurnal profile over a three day monitoring period in a general sample, not based on waist-hip ratio or psychological profile.

### **2.2.2 Patterns of Basal Diurnal Cortisol in High/Low WHR individuals in relation to Psychological & Metabolic Parameters**

Following on from the demonstration that it was indeed feasible to repeatedly sample cortisol in a free-living environment and that basal diurnal cortisol profiles are reproducible, one of the main aims of this thesis was addressed. Study One (Chapter Five) presents a detailed assessment of the potential differences in basal salivary cortisol activity between those who exhibit central obesity and those who do not. Salivary cortisol was assessed at set time points synchronised to waking over three consecutive week days. Additional factors that could potentially influence basal cortisol were assessed. These included sleep quality (using the LSEQ), daily hassles (using the Daily Hassles Scale), and perceived stress (using the PSS). Further, an assessment of a range of biological markers associated with obesity and the metabolic syndrome was made. These biomarkers included fasting plasma glucose, insulin (insulin resistance using the Homeostatic Model Assessment/HOMA technique), total cholesterol, triglycerides, high and low density lipoproteins, interleukin-6 (IL-6), C-reactive protein (CRP) and adiponectin.

### **2.3 Cortisol Responses to Stress in High/Low WHR Individuals**

Study Two developed the hypothesis that cortisol responses to stress are elevated in high waist-hip ratio individuals and investigated the cortisol response to stress using the Trier Social Stress Test (Kirschbaum et al., 1993). Differences in the cortisol response of high and low waist-hip ratio individuals were explored. Epel et al. (2000) assessed stress responsivity in a sample of females and found that increasing responsiveness was associated with central obesity. The study presented in Chapter Six, explored responses in both males and females. The hypothesis was that those who exhibit central obesity will show an elevated salivary cortisol response to a psychological stressor compared with lean or peripherally obese individuals.

### **2.4 The effect of Psychological Stress on Cognitive Performance in High/Low WHR Individuals**

The literature explored in Chapter One suggested that there may be links between cortisol, central obesity and cognition. Previous studies exploring the stress response in high waist-hip ratio individuals did not consider the impact this could have on cognitive performance. Further, central obesity has been identified as a risk factor for cognitive impairment in conjunction with other metabolic factors such as hypertension (Elias et al., 2005; Waldstein and Katzel, 2005). To date, the interaction of central obesity and cortisol on cognition has not been explored. The study presented in Chapter Six was an assessment of cognitive performance in high and low waist-hip ratio individuals and their cortisol response to stress following exposure to a psychological stressor. Chronic elevated cortisol has been shown to impact on cognitive performance, particularly declarative memory (Lupien et al., 2005). If those who exhibit central obesity also exhibit elevated cortisol in response to a psychological stressor, then one might predict poorer cognitive performance.



---

---

## CHAPTER THREE

### General Methodology

---

---

#### 3.1 Introduction

There is large variation between studies in terms of the methods employed to assess stress responsivity. Similarly, there is a large range of tests of cognitive function, each measuring change in one or more specific domains. While one might suppose that the classification of obesity has been agreed, this is not the case and there is an ongoing debate as to whether central obesity is better reflected in waist-hip ratio or waist circumference. Body mass index (BMI) is also subject to debate.

This chapter describes and discusses the methodology used to determine obesity in particular central obesity, the determination of cortisol and the resultant indices which can be calculated from the diurnal profile, as well as the stress induction technique (TSST) adopted in the studies presented in this thesis. Further, the use of the CANTAB neuropsychological assessment battery is explored. Additional measures employed in the three studies are described in their respective chapters.

#### 3.2 Measurement of Obesity

##### 3.2.1 Body Mass Index (BMI)

Body mass index (BMI) is a calculation based on height and weight, which provides an estimate of body mass. BMI is related to the risk of disease (Segal et al., 1986). A BMI within the 18-25kg m<sup>-2</sup> is considered normal. A BMI greater than 26 and less than 30kg m<sup>-2</sup> classifies an individual as being overweight and a BMI greater than 30kg m<sup>-2</sup> indicates obesity (NHLBI and NIDDKD, 1998) with a BMI of greater than 35 kg m<sup>-2</sup> indicating morbid obesity. A BMI greater than 30kg m<sup>-2</sup> is also indicative of risk of incidence metabolic syndrome (WHO, 2000). The relevance of body mass index as a marker of body composition is debated (Prentice and Jebb, 2001). BMI has been shown to provide misleading information about the presence of obesity in a range of

conditions, including ageing (Cohn, 1987), racial differences (Deurenberg et al., 1991; Rush et al., 1997) and athletes (Katch and Katch, 1984) leading to an incorrect diagnosis of obesity (Frankenfield et al., 2001).

### **3.2.2 Body Composition**

Due to the questionable reliability of body mass index alone, a measure of body composition using bioelectric impedance was made. The use of bioelectric impedance has been championed in the last decade in light of the failings of body mass index calculations (Houtkooper et al., 1996). Body composition (specifically fat mass) was hence determined via bioelectrical impedance analysis (BIA) using the 'Biospace In Body 3.0' body composition analyser. This gave an indication of the amount of fatty tissue, muscle and water that contributed to the overall weight of the person tested. This was conducted whilst the volunteer was in a fasted state.

### **3.2.3 Waist-Hip Ratio (WHR)**

As discussed in Chapter One, the measurement of central obesity can be conducted using more than one method, the most common being the measurement of waist to hip ratio. The World Health Organisation (WHO, 2000) defines central obesity as a waist-hip ratio of greater than 0.85 in females and 0.90 in males, a calculation based on accurate measurement of waist circumference compared to hip circumference. Several studies have demonstrated the effectiveness of measuring waist-hip ratio and its value in predicting negative health states (Hartz et al., 1984; Kalkhoff et al., 1983; Rivera and Svec, 1989). A single measurement of waist circumference has been used to predict morbidity, with a circumference greater than thirty-five inches in females or forty inches in males is indicative of central obesity (NCEP, 2001). However, Welborn et al. (2003) found that waist to hip ratio to be a more dominant risk factor for cardiovascular death than waist circumference alone.

When diagnosing central obesity, the distribution of fat is of paramount importance. Methods that consider whole body obesity may not be sensitive enough to determine central obesity from peripheral obesity. For example, body mass index (BMI) is a global measure of obesity, but is inaccurate in the diagnosis of central obesity. Kontogianni et al. (2005) found that BMI did not accurately reflect obesity status in a sample of perimenopausal women and found inconclusive evidence for the use of BMI in

determining vulnerability to obesity related disease. Skin fold thickness improves on BMI despite being an assessment of whole body obesity (using callipers). Blair et al. (1984) found similar results to waist-hip ratio measurements when using skin folds as a diagnostic tool for central adiposity when predicting later morbidity.

Other techniques for measuring fat distribution are available. Waist to stature ratio (WSR) proposes that waist circumference should not exceed half of the stature of the body. Ho et al. (2003) in exploring predictors for cardiovascular disease found this method to correlate more significantly than other measures (waist to hip ratio and waist circumference) with cardiovascular risk factors. However, WSR is a relatively new approach and as a result lacks reliability.

Other tools include the use of hip girth (Raja et al., 2004), 3D body scanning (Lin et al., 2004) and computerised tomography (e.g. Borkan et al., 1982 and Yoshizumi et al., 1999). Computerised tomography appears by far the most accurate method of measuring body fat and determining body fat distribution. Such an approach has been regularly applied to the diagnosis of Cushing's syndrome (Rockall et al., 2003), confirming the observation that patients with Cushing's syndrome accumulate visceral fat about the abdomen. However, computerised tomography despite its accuracy is impractical for research conducted outside of a clinical setting.

In this thesis, it was decided that waist-hip ratio was the most reliable measurement of central obesity when measured accurately. To ensure accurate measurement the following guidelines were adhered to in the studies presented in this thesis. Separate measurements of waist and hip circumference (in centimetres) were taken to determine body shape in terms of waist-to-hip ratio. Waist circumference was measured at the level midway between the lower rib margin and the iliac crest with volunteers in the standing position without heavy outer garments and with emptied pockets, breathing out gently. Hip circumference was recorded as the maximum circumference over the buttocks. Waist-hip ratio was subsequently calculated as the ratio of waist circumference over the hip circumference (Visscher et al., 2001; Molarius et al., 1999).

### 3.2.3.1 Obesity Related Biomarkers

The incidence of central obesity is commonly associated with an incidence of elevated blood biomarkers that are associated with poor health (Hartz et al., 1984; Kalkhoff et al., 1983). In particular central obesity concurs with the incidence of the metabolic syndrome (Bjorntorp, 1997). To assess the association between central obesity and poorer health a range of blood biomarkers were assessed that collectively form key metabolic syndrome symptomology. These are outlined below.

#### i. Glucose & Insulin (with calculated Insulin Resistance)

Blood plasma and serum were collected from a fasted blood sample to assess presence of diabetes / insulin resistance since these are key factors in metabolic syndrome symptomology in relation to the role of cortisol in the metabolic syndrome (See Chapter One, Section 1.5.2). The estimated degree of insulin resistance was inferred from the fasting blood glucose sample obtained via application of the Homeostasis Model Assessment (HOMA) technique. Concentrations of 11-12.2m $\mu$ /L or 6.1 – 7.0 mmol are indicative of impaired glucose tolerance (IGT). Increases in concentration of over 126mg/dl or 7.0 mmol/L indicate an increased risk of diabetes (Diabetes Mellitus Expert Committee on the Diagnosis and Classification of Diabetes, 1997). It is suggested that those with central obesity are at risk of impaired glucose tolerance and diabetes (Bjorntorp, 1997).

Fasting glucose and insulin measures were used to calculate the degree to which individuals may present insulin resistance. This was calculated using the homeostatic model assessment technique (HOMA) (Matthews et al., 1985). This provides an estimate of steady beta cell function and insulin sensitivity as a percentage of a normal reference population. The calculation is as follows:

$$\text{Insulin resistance (IR)} = \frac{\text{fasting plasma insulin} \times \text{fasting plasma glucose}}{22.5}$$

22.5

An assay for the detection of insulin was performed using a Perkin Elmer time resolved fluoroimmunoassay kit developed for use on AutoDelfia. The principle is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. Standards, controls and

volunteer samples containing insulin are reacted simultaneously with immobilised monoclonal antibodies directed against the insulin molecule and with europium-labelled monoclonal antibodies directed against different specific antigenic sites on insulin. This is carried out in one incubation step. Enhancement solution dissolves the europium ions from the labelled antibody, which produces fluorescence. This is measured in each well and the fluorescence from each sample is directly proportional to the concentration of insulin in the sample (Soini & Kojola 1983; Hemmila et al., 1984; Lovgren et al., 1985)

The analysis of volunteer samples to determine glucose concentration was conducted courtesy of Charing Cross Hospital, London using Hexokinase method on an Olympus AU640 analyser (reagents and analyser from Olympus Diagnostics, UK).

## **ii. Adiponectin**

Adiponectin has been found to be reduced in cases of obesity and type II diabetes. The level of adiponectin in serum has been found to be between 5 and 10  $\mu\text{g/ml}$  in human serum (Berg et al., 2001). This was measured in serum collected from a fasted blood sample to assess relationship between Adiponectin levels and the presence of insulin resistance.

An 'in-house' AutoDelfia assay at Colworth (Unilever Research and Development) was implemented to detect the level of adiponectin in human serum. The following protocol was adhered to. MAB10651 (antibody) was absorbed to the surface of each well of Nunc Maxisorb fluoronunc microtitre plates (100 $\mu\text{l}$  of 2 $\mu\text{g/ml}$  antibody in 50nM Bicarbonate buffer pH 9.8, overnight at 4°C). Sensitised plates were washed three times using PBST and 100 $\mu\text{l}$  serum samples or standard were added to the wells in duplicate (diluted 1/1000 in Perkin Elmer DELFIA assay buffer). Plates were incubated at room temperature for 1 hour on a microtitre plate shaker). The plates were washed as before and 100 $\mu\text{l}$  of the biotinylated detection antibody BAM1065 was added to each well (2 $\mu\text{g/ml}$  in DELFIA assay buffer). The plates were again incubated for 1 hour on a shaker. The plates were washed as before and 100 $\mu\text{l}$  of streptavidin Eu conjugate was added to each well (diluted 1/1000 in DELFIA assay buffer and filtered through a 0.22 $\mu\text{m}$  filter). The plates were again incubated for 1 hour on a shaker. The plates were given a final six washes and 200 $\mu\text{l}$  of enhancement solution was added to each well.

The plates were incubated for a minimum of five minutes on a shaker before reading via a Victor<sup>2</sup> multi-analyte detection system set up for the detection of free Eu.

### **iii. Total Cholesterol**

In addition to measured level of glucose and insulin, the measured level of total cholesterol has also been shown to be higher in those with central obesity. Elevated levels of total cholesterol (greater than 5.2mM/L) are indicative of high blood total cholesterol and risk of metabolic syndrome. Hence in the subsequent studies presented in this thesis, this was measured in serum collected from a fasted blood sample.

The analysis of volunteer samples to determine cholesterol concentration was conducted courtesy of Charing Cross Hospital, London. The assay employed; (i) The Cholesterol Oxidase method on an Olympus AU640 analyser (reagents and analyser from Olympus Diagnostics, UK) for cholesterol assessment. (ii) Enzymatic method on an Olympus AU640 analyser (reagents and analyser from Olympus Diagnostics, UK) for Triglyceride assessment. (iii) Immunoinhibition method on an Olympus AU640 analyser (reagents and analyser from Olympus Diagnostics, UK) for HDL cholesterol assessment and (iv) calculated from Total Cholesterol, HDL Cholesterol and Triglyceride using the Friedewald Equation  $\{LDL = TC - (HDL + TG/2.2)\}$  for LDL cholesterol assessment.

### **iv. High Density Lipoprotein (HDL) / Low Density Lipoprotein (LDL) Cholesterol**

In conjunction with total cholesterol, elevated LDL and reduced HDL are characteristic of central obesity and are additional symptoms of metabolic syndrome. Increased quantities of LDL (>3.5 mmol/L) indicate the possible presence of hypertriglyceridemia, in addition to low quantities of HDL (<1.1mM/L). Hence, this was measured in serum collected from a fasted blood sample to measure degree of hyperlipidemia as a potential marker for metabolic syndrome.

The analysis of volunteer samples to determine HDL and LDL concentration was conducted courtesy of Charing Cross Hospital, London using the same procedure as employed in the assessment of total cholesterol.

**v. Triglycerides**

Elevated levels of triglycerides ( $> 2.3\text{mM/L}$ ; WHO, 2002) are a risk factor for cardiovascular disease and diabetes and are markers for the metabolic syndrome. This was measured in serum collected from a fasted blood sample.

The analysis of volunteer samples to determine triglyceride concentration was conducted courtesy of Charing Cross Hospital, London using the same procedure as employed in the assessment of total cholesterol.

**vi. InterLeukin-6 (IL-6)**

Elevated levels of IL-6 are associated with the presence of metabolic syndrome. Levels of  $<1.0\text{pg/ml}$  are considered normal. This was measured in serum collected from a fasted blood sample to assess the concentration of circulatory inflammatory cytokines from blood samples to infer immune status.

The assay employed to determine IL- concentration employed the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-6 was pre-coated onto a micro plate. Standards and samples were pipetted into the wells and any IL-6 present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-6 was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added. After an incubation period, an amplifier solution was added and colour developed in proportion to the amount of IL-6 bound in the initial step. The colour development was stopped and the intensity of the colour measured.

**vii. C- Reactive Protein (CRP)**

In addition to an elevation of IL-6 in the metabolic syndrome, an additional inflammatory marker, CRP has also been shown to be elevated in a centrally obese state concurrent with metabolic syndrome. The normal range of CRP is between  $0.18$  to  $5.05\text{mg/L}$  (Tchernof et al., 2002). This was measured in serum collected from a fasted blood sample to assess immune function.

The analysis to determine CRP concentration was conducted courtesy of Charing Cross Hospital, London using Immunoturbidimetry on an Olympus AU640 analyser (reagents and analyser from Olympus Diagnostics, UK).

### **3.3 Measurement of Cortisol**

Research has used various psychological or biological indices of stress and stress responsivity ranging from subjective reports on psychological questionnaires to physiological biomarkers such as cortisol, the focus of the studies in this thesis.

#### **3.3.1 Cortisol**

##### **i. Detecting Cortisol**

Cortisol can be detected in human plasma, saliva and urine. Each method of detection has certain practical implications. For example, measuring urinary cortisol although useful for exploring cortisol excretion rate over the nocturnal period, is not appropriate for tracking rapid changes in cortisol activity (including the diurnal period) (Gozansky et al., 2005) as it takes a significant amount of time to excrete cortisol metabolites in urine. Plasma cortisol can track rapid changes in cortisol activity. However, the venepuncture procedure artificially elevates cortisol activity (Meeran et al., 1993) as it can be a stressful procedure. Further, serial blood collections for the assessment of cortisol are impractical, uncomfortable and inconvenient for the volunteer. The use of saliva sampling overcomes these practical issues. Samples are quick and easy to collect using specialised collection devices (salivettes). Cortisol in saliva is able to track rapid changes in activity. Further, salivary cortisol reliably and accurately correlates with plasma cortisol when analysed using a radioimmunoassay (RI) procedure (Vining et al., 1983). This applies to the assessment of basal cortisol, cortisol responses to an external stressor and responses to exogenous glucocorticoid administration (Umeda et al., 1981; Kahn et al., 1988; Port, 1991; O'Connor and Corrigan, 1987; Vining et al., 1983; Contreras et al., 2004 and Turin et al., 1992). Gozansky et al. (2005) contributed additional support for the use of salivary cortisol using a commercial enzyme immunoassay (EIA) to confirm that salivary cortisol analysis represents the bioactive fraction of cortisol (not the cortisol that is bound to cortisol binding globulin (CBG) or other proteins) compared to serum cortisol. This accurately reflects the activity of the hypothalamic-pituitary-adrenal (HPA) axis. Hence, Gozansky et al. (2005) argued that



the use of saliva for cortisol analysis is physiologically more relevant in stress research. Further, Putignano et al. (2002) demonstrated the effectiveness of salivary cortisol assessment in exploring differences in cortisol activity in those demonstrating central obesity.

In this thesis, the collection of saliva for cortisol assessment was deemed to be an acceptable method for exploring differences in cortisol activity between individuals with central obesity and lean or peripherally obese individuals, for both the diurnal profile and stimulated cortisol responses to stress.

## **ii. Sampling Methods**

The number and frequency of saliva samples collected for the assessment of basal cortisol varies largely in research studies. Early research formed conclusions about cortisol awakening activity based on one saliva sample obtained during the early morning (Simkin et al., 1961; Szenas and Pattee, 1959). This was later deemed insufficient to accurately assess the dynamic activity of cortisol during the period of the cortisol awakening response (Hucklebridge et al., 1998; Pruessner et al., 1997). Consequently, it is now accepted that a series of samples are required. For example, a number of studies have obtained samples immediately upon waking, 30minutes, forty-five minutes and sixty minutes post waking (Federenko et al., 2004; Wust et al., 2000). Other studies have conducted a more detailed sample collection procedure and obtained samples at waking, 15minutes, 30minutes, 45minutes post waking and every three hours post waking until 12hour post waking in an attempt to capture more sensitive changes in the cortisol awakening response and subsequent diurnal response (Edwards et al., 2001;2001; Hucklebridge et al., 2002; 2005). Some studies have obtained fewer samples, collecting samples upon waking and subsequently at thirty and sixty minutes (Williams et al., 2005) or at waking and 30minutes post waking (Kunz-Ebrecht et al., 2004) hence there is some variation in terms of capturing the cortisol awakening response. In this thesis, a more detailed profile of cortisol activity over the diurnal period was required. Hence, the sample times adopted by Edwards et al. (2001) and Hucklebridge et al. (2002; 2005) were used as a guide, that is to say that samples were collected immediately upon waking (0minutes), 15, 30 and 45 minutes post waking and subsequently at 3, 6, 9 and 12 hours post waking.

It is also important to consider the period of saliva sampling. For example, Steptoe et al. (2004) obtained saliva samples over the course of one day only. Other studies have monitored cortisol activity over two days (for example, Edwards et al., 2001; Federenko et al., 2004) and one study assessed cortisol activity over a six day period exploring differences between responses on week days to weekends (Schlotz et al., 2004). Cortisol responses appear to differ between weekdays and weekends (Kunz-Ebrecht et al., 2004; Thorn et al., 2006) with attenuated cortisol awakening responses at the weekend compared to weekday. In the current study it was decided to monitor the activity over cortisol over a three day weekday period in order to obtain an aggregate profile of cortisol activity to improve accuracy of the cortisol responses collected and gain a more detailed view of the consistency of cortisol activity within each volunteer over a three day period. To prevent extraneous factors altering measured cortisol awakening responses, sampling over a weekend was not permitted.

The nature of waking can also influence subsequent changes in cortisol output. Some studies adhere to a pre-decided 'clock-time waking' rather than natural biological waking time (Ice et al., 2004; Smyth et al., 1997) with actual time points synchronised to this time of waking (e.g. 8am). Other studies have explored the collection of saliva samples that are synchronised to natural waking followed by set time points based on time of waking (Abercrombie et al., 2004). Both approaches have observed methodological issues. A forced waking time may not be truly representative of the natural cortisol response to waking leading to an alteration of the cortisol awakening response (Spath-Schwalbe et al., 1991). Natural waking, with waking time synchronised sampling, is heavily reliant on the compliance of the volunteer and may result in a lack of synchronicity and inaccurate data being obtained. However, while it is possible to screen data for non-compliance once obtained, it would be difficult to ascertain whether the cortisol awakening response was altered due to forced waking. For this reason, responses to natural waking with set time points after this were selected as the method used in this thesis.

In this thesis, eight Sarstedt salivettes were provided for each volunteer per day sampled; each was labelled clearly with the sample day and time. These samples were taken immediately upon waking, 15mins, 30mins and 45mins post waking, and subsequently at 3, 6, 9 and 12 hours post waking. The volunteers were provided with

both oral and written instructions on how to use the salivettes correctly. It was imperative that the cotton wool insert be kept in the mouth for an absolute minimum of thirty seconds and ideally retained in the mouth for one minute to allow to complete saturation of the cotton wool with saliva. The volunteers were asked to refrain from eating and drinking or from brushing teeth until the samples for the assessment of the cortisol awakening response had been collected. This is due to the observation that vascular leakage and micro-abrasion can alter the saliva in the sample (Vining and McGinley, 1987). Once returned to the laboratory, the salivettes were frozen at  $-20^{\circ}\text{C}$  by the researcher to precipitate mucins until transported for analysis. The data was used to construct a cortisol diurnal profile for each day incorporating the cortisol awakening response to assess consistency over the sampling period with a view to forming an aggregate profile to compare between groups

### **iii. Biochemical Assay of Saliva for Cortisol**

A number of assay techniques exist that may be employed to determine the concentration of cortisol in saliva. The majority of previous studies employ a radioimmunoassay (RIA) technique (e.g. Cook et al., 1997; Morineau et al., 1997). Another common technique is the use of the widely available Salimetrics enzyme immunoassay (EIA) kit (Salimetrics, LLC) specifically developed for use with saliva. Cortisol was determined in the studies presented in this thesis by a DELFIA validated 'in-house' assay developed at Unilever Research and Development, Colworth, that was more cost effective in comparison to the Salimetrics EIA and equally as sensitive and reliable. The amount of cortisol present in the saliva samples was determined using a competitive inhibition immunoassay using fluorescence on AutoDelfia at Colworth (Unilever Research and Development). This was an in-house technique developed from previous use of the widely used Salimetrics Kit (Salimetrics LLC). The process uses pre-coated and quality controlled Goat anti-Rabbit plates (rabbit cortisol antibodies linked to goat peroxidase). Cortisol in the unknown samples competes with the cortisol contained on the plate for the antibody binding sites during an incubation period. Unbound components are washed away and the plate is treated with europium treated cortisol (Cortisol  $\text{Eu}^{3+}$ ) as a fluorescence to illuminate the degree of binding and determine to concentration of cortisol present in the sample (by 'counts'). The count of cortisol europium binding is inversely proportional to the concentration of cortisol present.

### 3.3.2 Dehydroepiandrosterone (DHEA)

DHEA is related to cortisol and can be readily measured in human saliva (using passive drool samples). DHEA demonstrates a circadian rhythm in a similar profile to cortisol that has been shown to diminish with age and appears unaffected by cortisol circadian activity (Ceresini et al., 2000). More importantly, DHEA has often been linked to cognitive performance via its relationship with cortisol. Cortisol and DHEA are linked as a ratio of action. Research suggests that a low DHEAS: high Cortisol ratio leads to cognitive impairment particularly in elderly people (Kalmijin et al., 1998). An assessment of dehydroepiandrosterone (DHEA) was made via collection of passive drool samples using a small pre-labelled collection pots. One awakening sample was obtained was collected per day of assessment in Chapter Five and Chapter Six. Samples were obtained immediately upon waking in conjunction (but not following) the first cortisol sample. Salivary DHEA correlates well with plasma DHEA ( $r = 0.9$ ; Goodyer et al., 1996) and shows stability over a two day sampling period (Hucklebridge et al., 2005).

The assay procedure for determining Dehydroepiandrosterone concentration present is similar to the procedure for cortisol analysis in that it is based on the original Salimetrics Kit technique and uses horseradish peroxidase coated plates, again with rabbit antibodies. Following incubation, the unbound components are washed away, the degree of bound DHEA peroxidase is determined by the reaction of the peroxidase enzyme on the added substrate tetramethylbenzidine (TMB). This reaction produces a blue colour. The reaction is stopped by addition of sulphuric acid changing the colour from blue to yellow. The optical density is subsequently read on a plate reader. The amount of DHEA peroxidase present is inversely proportional to the concentration of DHEA present in the sample.

### 3.3.3 Assessing Cortisol Diurnal Profiles (Computational Indices)

There are a number of techniques that can be applied in the statistical assessment of the basal diurnal cortisol profile. As previously, discussed, salivary samples for cortisol assessment are usually collected over a period of 1-3 days. Past research has formed an aggregated profile in determining average cortisol activity over a brief period (1-3 days) and on occasion assessed the consistency of the profiles obtained (e.g. Edwards et al., 2001). Assessing profile consistency has been found to be particularly useful in

exploring HPA regulation (Ice et al., 2004; Smyth et al., 1997). An inconsistent profile may reflect poor HPA regulation. Another approach to the data is the use of calculated indices of cortisol activity. These indices reflect changes in the rate and level of cortisol secretion at certain time points across the diurnal period including change in cortisol during the cortisol awakening response (CAR). These indices are outlined below:

**i. Area under the curve with respect to zero (AUC) (Edwards et al., 2001)**

The AUC is an assessment of the amount of cortisol secreted during the awakening response from 0 (Sample A), 15 (Sample B), 30 (Sample C) and 45minutes (Sample D) post waking. This is calculated as follows:

$$\text{Sample A} + \text{Sample B} + \text{Sample C} + ((\text{Sample D} - \text{Sample A}) / 2)$$

**ii. Area under the curve with reference to the first sample (AURC) (Edwards et al., 2001).**

The AURC is an assessment of the amount of cortisol secreted during the awakening response from 0 - 45minutes post waking. This was calculated as follows:

$$\text{Sample B} + \text{Sample C} - (2 * \text{Sample A}) + ((\text{Sample D} - \text{Sample B}) / 2)$$

**iii. Mean Increase in cortisol from waking to 45 minutes post waking (MnInc) (Edwards et al., 2001)**

The MnInc is an assessment of the rate of increase in cortisol activity from 0 - 45 minutes post waking. This was calculated as follows:

$$(\text{Sample B} + \text{Sample C} + \text{Sample D}) / 3 - \text{Sample A}$$

**iv. Change between waking (Sample A) & 30minutes (Change 0-30) (Steptoe et al., 2004).**

Change 0-30 is an assessment of the change in cortisol activity from 0 - 30minutes post waking to examine the cortisol response to waking. This analysis has been used recently in research that specifically explores associations between cortisol and central obesity.

**v. Difference between the awakening cortisol level & the final sample (Day-Difference, Edwards et al., 2001)**

Day-Difference is an assessment of the change in cortisol level between waking and the 12-hours post waking. This reflects the rate of cortisol activity over the course of the diurnal profile.

**vi. Difference between the 3h sample & 12hr sample (day Difference 3-12; Edwards et al., 2001)**

The Day-Difference index can also be applied to assess cortisol activity over the diurnal period excluding the cortisol awakening response as a change between 3 hours post waking and 12 hours post waking.

**vii. Final Sample (Sample H) (Edwards et al., 2001)**

Assessment of the Final Sample has often been used as an indicator of continuing cortisol activity into the evening/nocturnal period after the 12-hour assessment has completed. It is postulated that an elevated final sample indicative of the possibility of elevated cortisol secretion throughout the evening and nocturnal period and can be a predictor of subsequent health problems (Dallman et al., 1993).

**viii. Day Mean (Edwards et al., 2001)**

Day Mean, using equal interval sample times (0, 3, 6, 9, 12) can be calculated to assess the mean level of cortisol activity across the diurnal profile.

**ix. Diurnal Mean**

A Diurnal Mean in addition to a Day Mean (previous) can be calculated using the diurnal sample time points E, F, G and H (3hour – 12hours post waking) as an indication of the mean level of cortisol activity post awakening response.

### **3.4 Biopsychological Stress Induction & Measurement**

#### **3.4.1 Stress Induction: The Trier Social Stress Test**

The Trier Social Stress Test (TSST) (Kirschbaum et al., 1993) combines a public speaking (interview) with a mental arithmetic task in front of a panel of judges. Application of the TSST as a stress induction tool successfully elevates ACTH, growth

hormone, prolactin, and heart rate post administration (Kirschbaum et al., 1993). A 2- to 4- fold increase in cortisol (both serum and saliva) from baseline has been observed in 80% of subjects who undergo the TSST (Schommer et al., 2003) with subjects displaying at least a 2.5mmol/l increase in cortisol as a result of exposure (Kirschbaum et al., 1993). The TSST has applications in a wide range of research but with particular relevance in the investigation of stress induced memory impairment (Domes et al., 2002; Hoffman and Al'Absi, 2004; Lupien et al., 1997; Maheu et al., 2005; Takahashi et al., 2004) where the impact of stress-induced cortisol elevations on cognitive performance was assessed.

The procedure adopted for the TSST is consistent among previous applications. The success of the TSST is hinged upon the inclusion of a socio-evaluative threat component with an element of uncontrollability (reviewed by Dickerson and Kemeny, 2004). This combination is most effectively represented in a public speaking task. The TSST adopts the following procedure. The volunteer is taken into a room (a) where they are introduced to the task they were about to complete. In a separate room, (b), two people are sat behind a desk (interview panel) with a video camera and microphone installed. The volunteer is taken into room (b) and asked to sit in front of the panel (the panel always comprises both male and females). The volunteer is asked to assume the role of a job applicant who has been invited for a personal interview with the company staff managers (the selection committee). These managers are introduced as being especially trained in the assessment of non-verbal behaviour. The volunteer is informed that following a brief preparation period, they will be asked to introduce themselves to the panel and perform a 5 minutes speech detailing why they feel they are the best person to fill the vacancy. Further, the volunteer is informed that a voice frequency analysis of their performance is to be conducted. Following these instructions, the volunteer is returned to room (a) where they are allowed 10 mins preparation for their speech. Pen and paper notes are permitted whilst in preparation but are not allowed in the interview room.

After ten minutes of preparation, the volunteer is taken back into room (b), whilst the investigator waits outside. The manager invites the volunteer to begin the speech and the volunteer stands before the committee. If the volunteer has finished his speech prior to the allotted five minutes, the managers respond using a standardised script. First, the

volunteer is told, "You still have some time, please continue!" If the volunteer stalls again before the end of the five minutes, the managers remain silent for 20 seconds before using a set of prepared questions.

Fifteen minutes into the TSST, the volunteer is instructed to serially subtract the number 13 from 1022 as fast and accurately as possible. On every failure, the volunteer is asked to restart, by the command "stop 1022" (this is a timed five-minute task; however, the volunteer is unaware of the time allotted). Twenty minutes into the TSST, the task is complete and the volunteer is taken back into room (a). The volunteer is fully debriefed at the end of the experiment and is informed that neither voice pattern nor video analysis was performed.

Saliva samples for cortisol assessment were collected at baseline and throughout the TSST procedure (six samples in total). Concurrent with cortisol samples, blood pressure was also measured using an ambulatory blood pressure device (Star Labs®). To measure subjective stress, the state trait anxiety inventory (STAI) and state self esteem scale (SSES) were administered at baseline and subsequently at set time points during the procedure. At the end of the session, a debrief questionnaire in the form of visual analogue scales was administered to assess subjective perception of how stressful the TSST was perceived to be (See Section 3.4.2.3).

### **3.4.2 Subjective Measures of Stress**

#### **3.4.2.1 Perceived Stress Scale (PSS; Cohen, 1994).**

The PSS is a widely used, reliable tool, which is a measure of the perception of stress and the degree to which situations in an individual's life are appraised as being stressful (See Appendix 10). The questions contained are easy to understand, easy to respond to and are free from context specific effects to any subpopulation. The short form of the perceived stress scale (used in this thesis), also allows for quick administration. Responses are made on a five point Likert Scale (0-4) from "never" to "very often" with a higher score suggesting greater perceived stress. Scoring is based on norms for defined categories including gender, age, and race. Scores above 12.1 in males and 13.7 in females are considered above average indicating greater subjective stress. Assessment is usually made from thoughts over the past month but can be adjusted for weekly or daily assessment and so is flexible in its administration. In this thesis, a



monthly assessment was made for a more general perception of stress. Assessment of the reliability of the Perceived Stress Scale yields satisfactory internal consistency with Cronbach alpha's ranging from 0.84 to 0.86 (Cohen et al., 1983). Similarly, no ceiling or floor effects have been observed (Froelicher et al., 2004). This measure was administered prior to the start of the study to assess how stressful each volunteer perceived their current situation to be. This was of particular relevance when exploring basal cortisol diurnal profiles. A non-classic diurnal profile may be attributed to greater subjective perceived stress.

#### **3.4.2.2 Daily Hassles Scale (Kanner et al., 1981)**

As an additional measure of daily stress, the Daily Hassles Scale was administered in conjunction with the PSS. The subjective reporting of daily hassles could impact heavily on the expression of basal diurnal cortisol profiles. The Daily Hassles Scale consists of 117 items that cover aspects of health, family, friends and the environment, practical considerations and chance occurrences (See Appendix 15). These items measure the frequency and severity of transactions with the environment that are considered by the person to be stressful. Volunteers rate each item on a scale of 0 (did not occur), 1, 2 or 3 (somewhat, moderately or extremely). Scores include an indication of the severity of the hassle and its persistence (yielding essentially the same information  $r = 0.95$ ; Kanner et al., 1981). A frequency score is obtained by counting the number of hassles checked. An intensity score is calculated by taking the mean of the severity rating. In this thesis, the scale was amended to include a self-report section for the volunteer to detail particular events experienced during sampling that were perceived as being particularly stressful. Test re-test assessment based on monthly administration demonstrates consistency of scores. Correlations are higher for frequency (average  $r = 0.79$ ) than intensity of hassles (average  $r = 0.48$ ) (Kanner et al., 1981). Themes have been identified according to age for the most frequently checked hassles. For example, middle-aged volunteers tend to select hassles related to property, finance, taxes etc.

#### **3.4.2.3 Visual Analogue Scales (Debrief Questionnaire)**

At the end of the test session in Chapter Six, an 'in-house' debrief questionnaire using visual analogue scales was administered to assess perception of the stress induction procedure (See Appendix 8). The questions assessed perceived preparation,

performance and stressfulness of both the TSST and also of the cognitive test battery (CANTAB). It has been suggested that individual response to a stressor is largely dependent on the perception of the stressor. If a stimulus is not perceived as being stressful then little or no response will be observed (Holroyd and Lazarus, 1982; Levine, 1978; Vogel, 1985).

### **3.4.3 Additional Physiological Measures of Stress**

#### **3.4.3.1 Blood Pressure**

This was measured at the screening stage of the study using an ambulatory blood pressure device (Omron ®) to assess potential hypertension as a component of the metabolic syndrome. A measured blood pressure of over 160 mmHg / 90 mmHg indicates the risk of hypertension (WHO, 2000) and was measured prior to experimental completion. Blood pressure exceeding 140/90mmHg is indicative of risk of incidence of Metabolic Syndrome (WHO, 2000). The pressure cuff is placed on the preferred arm (usually the left). An initial reading is taken to demonstrate the procedure to the volunteer (this reading is later discarded). The volunteer is asked to undergo seated rest for 5-minutes whilst wearing the blood pressure cuff to acclimate to the equipment. Four readings subsequently taken at 1 minute intervals whilst the volunteer remains seated (Bardwell et al., 2000; Everson et al., 2001) and an average of these readings was taken.

During the test session a continuous measurement of blood pressure was taken using an ambulatory device (Star Labs ®). This was taken to assess the physiological response to stress in conjunction with measures of cortisol and psychological appraisal using the STAI and SSES. Blood pressure responses to stress have been found to correlate with stress –induced changes in cortisol (Cacioppo et al., 1995).

### **3.5 Measuring Cognitive Performance**

A wide range of cognitive testing procedures have been implemented in research exploring stress-induced cognitive impairment. These range from those testing a wide range of cognitive performance in the form of a battery (e.g. Hoffman and Al'Absi, 2004) to specific tests of declarative versus non declarative memory using simple immediate and delayed word recall lists (e.g. Lupien et al., 1997). In this thesis, it was

deemed appropriate to administer a range of cognitive tasks due to the observation that impairment, although more commonly observed for declarative memory (Lupien et al., 2005), has also been observed on other tasks, for example spatial memory (Young et al., 1999) and attention (Bohnen et al., 1990). Therefore, the Cambridge Automated Neuropsychological Test Battery (CANTAB) was supplemented with a specific test of immediate and delayed verbal declarative memory, the auditory verbal learning task (AVLT) (Rey, 1964).

### **3.5.1 Background**

Volunteers were assessed on their cognitive performance using the Cambridge Neuropsychological Test Automated Battery (CANTAB). Originally developed at the University of Cambridge by Professor Robbins and colleagues in 1986, the battery has gained considerable popularity in the assessment of functional deficit related cognitive impairment. The battery is sensitive to a range of brain disorders and has been applied in the assessment of patients with Parkinson's disease, Alzheimer's disease, unipolar depression and schizophrenia. CANTAB is language independent with good test re-test reliability and clinical validity through extensive application in clinical research. For example in brain lesion studies (e.g. Fowler et al., 1997), degenerative disorders e.g. Parkinson's disease (Owen et al., 1997) and psychiatric illness (Rahman et al., 1999). The accuracy and sensitivity of CANTAB has also been confirmed from neuroimaging tests (PET and fMRI) in patients exhibiting brain dysfunction (Lee et al., 2000). Sensitivity to cognitive deficit associated with human ageing has also been demonstrated (De Luca et al., 2003; Rabbitt and Lowe, 2000). The range of level of difficulty within the 13 subtests prevents floor and ceiling effects and allows for a more accurate assessment of cognitive performance.

### **3.5.2 Cognitive Tasks**

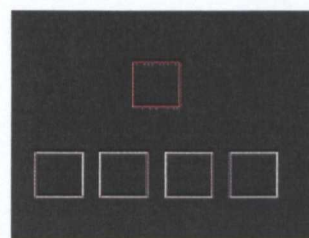
The battery contains a number of neuropsychological tests of which eight were selected for the current study and are detailed below. The tests were administered using a touch screen computer with the researcher present, to guide the volunteer through the correct procedure. The tests are detailed in the following sections:

**(a) Auditory Verbal Learning Task (AVLT) (Rey, 1964)**

The volunteer is verbally presented with a list of 15 words ‘List A’ and asked to listen very carefully (See Appendix 16). When the researcher finishes reading the list, the volunteer is asked to repeat as many of the words back to the researcher from the list as they can remember. The volunteer is not informed of the number of correct response, any repeats made or of the number of words yet to recall. Any repeats are noted using appropriate documentation in addition to any word confusions/associations or confabulations. This is repeated for five trials, for each trial the volunteer is asked to predict the number of words they estimated that they would be able to recall. Following trial five, a new list ‘List B’ is introduced to the volunteer and the same procedure repeated for one trial. The volunteer is asked to begin the CANTAB test battery. Following the paired associates learning task (PAL) the volunteer is asked to recall verbally as many words as possible from List A (after a delay of approximately 20minutes). All words recalled are recorded in addition to any errors or any intrusions from List B. Care was taken to ensure that neutral words were included in the word lists to avoid the influence of emotion on stress-induced verbal recall (See Chapter One, Section 1.6.4).

**(b) CANTAB****i. Motor Screening (MOT)**

This task was administered before all other tests as part of a training task to ensure accurate use of the touch screen and assesses the possibility of any visual or movement problems. The volunteer is asked to touch a series of crosses that appear on screen randomly so that they disappear as quickly as possible (Figure 3.1). The researcher first demonstrates the procedure.

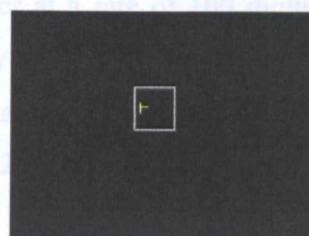
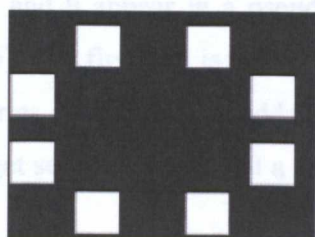
**Figure 3.1:** Motor Screening**Figure 3.2:** Delayed Matching to Sample

### ii. Delayed Matching to Sample (DMS)

The volunteer is shown a complex visual pattern (sample) and after a brief delay, four patterns (Figure 3.2). Each pattern is made up of four sub elements each in a different colour. One of the four choices is identical to the sample, one is a novel distracter, one has the shape of the sample but the colours of the distracter and one has the colours of the sample but the shape of the distracter. To avoid the possibility of a strategy based on learning single quadrants, all four choices have one quadrant in common with the sample. The volunteer is required to correctly identify the sample from the four choices by touching the selected pattern on the screen. If the volunteer makes an incorrect choice, then a second choice must be made and so on until the correct pattern is identified. The task begins with three practice trials (aided by the researcher) followed by 20 counterbalanced trials of simultaneous, 0, 4 or 12 second delay presentation.

### iii. Paired Associates Learning (PAL)

The task requires the volunteer to remember patterns associated with different locations on the screen. Six boxes appear on the screen and are opened randomly revealing a pattern (Figure 3.3). During the test phase, the volunteer must point to the correct location as each pattern is presented. The test moves through varying levels of difficulty from 2 locations through to 8 locations. If an error is made, the patterns are presented again and the volunteer must repeat the trial. The volunteer can have up to ten repeat reminder trials within each set of patterns. If the trial is not completed within those ten repeat trials, the test is automatically terminated. This task tests the ability to form visuospatial associations within a delay response procedure. In particular two aspects are assessed; 'list memory' indicating the correct identification of the patterns in their locations and 'list learning' in reference to the number of times the patterns are repeated in order to learning to occur, leading to a correct identification.



**Figure 3.3:** Paired Associates Learning Task

**Figure 3.4:** Pattern Recognition Memory

#### iv. Pattern Recognition Memory (PRM)

The volunteer is presented with a series of visual patterns (12 in total) in the centre of the screen (Figure 3.4) and asked to remember them. These patterns are designed so that they cannot be assigned a verbal description. In the recognition phase, the screen displays two patterns, one is the test pattern, and one is a distracter. the volunteer is required to select the pattern they believe was part of the list previously viewed by touching the screen. In this phase, the test patterns are presented in reverse order to the original order of presentation. This is repeated with 12 new patterns.

#### v. Spatial Recognition Memory (SRM)

The volunteer is presented with a white box that moves about the screen in a set sequence to five different locations (Figure 3.5). In the recognition phase five pairs of boxes are presented on the screen, one is the target and one is a distracter. The volunteer is asked to indicate by touching the screen which box is in a location previously viewed in the presentation phase. This test is repeated three times each with five new locations.

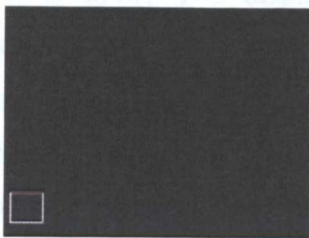


Figure 3.5: Spatial Recognition Memory



Figure 3.6: Rapid Visual Processing

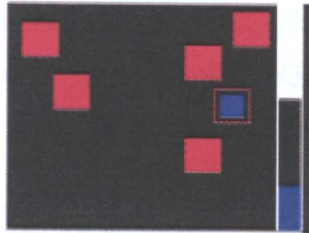
#### vi. Rapid Visual information Processing (RVP)

This task is an assessment of sustained attention with a small working memory component. A white box appears in the centre of the screen, inside of which numbers between 2 and 9 appear in a pseudo-random order at a rate of 100 digits per minute (Figure 3.6). The first trial is a practice followed by a test trial. The volunteer is asked to detect a series of consecutive odd numbers 3, 5, 7 and even numbers 2, 4, 6 by a button press. Target sequences occur at a rate of 16 every 2minutes.

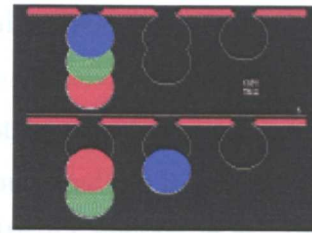
#### vii. Spatial Working Memory (SWM)

The task set is to find the blue token within each of the available boxes and use them to fill up an empty column at the side of the screen (Figure 3.7). The number of boxes

displayed on the screen increases to a total of eight. The colour and location of the boxes changes with each trial to deter strategy formation. The volunteer must open each box in turn until one reveals a blue token. The token is transferred to the column at the side of the screen. The box that contained the token will no longer hold a future token and opening this box again is recorded as an error. The volunteer continues to search for the remaining tokens without re-visiting boxes that have already contained tokens.



**Figure 3.7:** Spatial Working Memory



**Figure 3.8:** Stockings of Cambridge

#### viii. Stockings of Cambridge (Tower of London) (SOC)

This task is a spatial planning test that is based on the Tower of London task. The subject is shown two displays containing three coloured balls (Figure 3.8). The volunteer must use the balls in the lower display to copy the pattern in the upper display. The balls can be moved one at a time by touching the required ball and then the position to which it is to be moved. The time taken to complete the pattern and the number of moves are recorded as an indicator of the volunteers planning ability (there is no time limit). At first only one ball is to be moved, this increases gradually to five moves. Two task insertions are made of a procedure to control motor performance. If the volunteer makes more than double the number of moves for a trial, the trial is terminated. If three trials in a row are terminated then the task is terminated.

#### 3.5.3 National Adult Reading Test (NART, Nelson, 1982)

Chapter Six conformed to a between subjects design. This was adopted primarily due to the potential for the TSST to evoke anticipatory responses in a within subjects design which could create unnecessary noise in the data set. In order to control for differences between volunteers in terms of cognitive ability, the National Adult Reading Test (NART) was administered to provide an estimate of IQ of which could be used as a covariate in the subsequent analysis of cognitive performance to control for prior differences in cognitive ability.

The NART is an estimation of premorbid intelligence. The test consists of 50 words of increasing difficulty of which volunteers are asked to read and pronounce (See Appendix 14). The words included on the scale deviate from the standard grapheme-phoneme and stress rules of pronunciation. Therefore, in order for a volunteer to correctly pronounce a word, they must first be familiar with it. The number of mispronounced words is recorded to produce an error score. This is entered into an equation ( $128 - 0.83 \times \text{NART error score}$ ) to calculate estimated WAIS (Wechsler Adult Intelligence Scale) full scale IQ. The NART is successful in satisfying three criteria for the viable estimation of IQ. Firstly, the test is reliable, second, it is capable of predicting a substantial degree of variance in IQ and finally it is resistant to pre-existing neurological or psychiatric disorder (Crawford, 1989; Crawford et al., 1990; O'Carroll et al., 1992).

### **3.6 Conclusions**

To examine the aims outlined in Chapter Two, it was necessary to identify suitable methodologies and appropriate tests for the assessment of the three main areas of interest; cortisol, central obesity and cognitive performance. Although these techniques have not been employed in combination in the investigation of the effects of stress in high and low waist-hip ratio individuals, the evidence for their sensitivity has been demonstrated in the studies reviewed in Chapter One. The methods outlined in this chapter, were chosen as they were considered to be the most reliable and valid of those available for the studies outlined in this thesis.



**CHAPTER FOUR****The Practicalities of Assessing Basal Salivary Cortisol:  
A Pilot Study**

---

---

**4.1 Introduction**

Cortisol demonstrates a clear circadian profile of activity over the course of 24-hours (Born et al., 1999). The natural circadian activity of cortisol can be displayed graphically in the form of a diurnal profile. This profile is a plot of basal salivary cortisol concentration against time post waking and includes the cortisol awakening response (CAR). A dramatic change in cortisol activity is observed, during the first 30-45minutes post waking (Born et al., 1999; Hucklebridge et al., 1998; Prüssner et al., 1997), with an average increase of 9nmol/l within a range of 4-15nmol/l (Clow et al., 2004). Plotting a diurnal profile permits assessment of the cortisol awakening response in the continuous measurement of cortisol activity during the afternoon and evening (Stone et al., 2001). Cortisol diurnal profiles can be compared in terms of a number of calculated cortisol indices (Edwards et al., 2001; Schmidt-Reinwald et al., 1999; Wüst et al., 2000a) (see Chapter Three, Section 3.3.3). These are calculations based on the shape of the cortisol profile obtained and indicate the overall level of cortisol activity during a day. There are a number of practical issues to consider when sampling basal salivary cortisol. Previous studies differ in the number of days sampled to observe typical diurnal activity. For example, Steptoe et al. (2004) obtained saliva samples over a single day. Other studies have assessed basal cortisol over two days (Federenko et al., 2004; Edwards et al., 2001). The assumption that a single day of monitoring is sufficient to reflect typical activity will be addressed. Further the suggestion that free-living studies are commonly associated with non-compliance (Kudielka et al., 2003) despite high ecological validity will be explored.

## **4.2 Objectives**

This study aimed to explore the cortisol diurnal profile in an older adult sample (35-65). Profiles were explored for consistency over a period of three consecutive sampling days to determine the reproducibility and reliability of the cortisol diurnal profile. Further, the feasibility of implementing an averaging process on which the calculated cortisol indices are based was assessed. These indices were used to compare the profiles obtained over the three sample days. The practicalities of using saliva sampling as a means of cortisol assessment were explored.

## **4.3 Method**

### **4.3.1 Sample**

Volunteers were recruited from the Institute of Psychological Sciences, University of Leeds via an email recruitment flier to staff within the department. Eight volunteers were recruited to complete a three consecutive day period of saliva sampling (5 females, 3 males). All were aged between 35 and 65 with an average age of  $50.25 \pm 6.1$  years. Smokers and individuals using any form of steroidal medication were excluded.

### **4.3.2 Design**

The study conformed to a repeated measures design with each volunteer completing a three consecutive day period of saliva sampling with 8 within day observations (cortisol samples). A correlational assessment of each calculated index of cortisol activity across the three sampling days was conducted.

### **4.3.3 Measures**

#### **4.3.3.1 Psychological Measures**

Potential volunteers were asked to complete a screening booklet which was designed to collect background/demographic information (height, weight, marital, occupational and health status – See Appendix 1) and to administer the Perceived Stress Scale (PSS; Cohen, 1994) to measure subjective stress (see Chapter Three, Section 3.4.2).

#### **4.3.3.2 Physiological Measures**

Physiological measures of blood pressure, height and weight (to calculate BMI) and waist-hip ratio (WHR) were obtained to assess body shape and current health status (see Chapter Three, Section 3.2).

#### **4.3.3.3 Biological Measures (Cortisol)**

Salivary cortisol was measured eight times over each day for three consecutive days using pre-labelled salivettes (Sarstedt) for each sample day and time. Twenty-four salivettes per volunteer in total were collected.

#### **4.3.4 Procedure**

The procedure for the collection of cortisol and biochemical assay was described in Chapter Three (see Section 3.3). Further to this, the following procedure was followed on each day of saliva sampling.

Volunteers were asked to refrain from the consumption of alcohol the night prior to the experiment commencement since alcohol can falsely elevate cortisol levels on the subsequent sampling day. The following protocol was adhered to:

- i. **Immediately** upon waking the volunteer was required to produce an initial cortisol sample using the designated salivette provided. The volunteer was asked to remain in a supine, relaxed position and to abstain from food and drink.
- ii. The next sample was taken 15 minutes post waking and step (ii) repeated using the designated salivette provided.
- iii. The same procedure was repeated at 30 minutes and 45 minutes post waking. The volunteer was asked to remain in a supine position until after the 30-minute sample. Volunteers were asked to refrain from the consumption of food or drink other than water until after the 45-minute sample. Similarly, the volunteer was asked to refrain from brushing their teeth until this sample had been provided (to avoid vascular leakage and micro-abrasion, which has been shown to alter the saliva in the sample).

iv. Volunteers were asked to continue the saliva samples over the course of the afternoon. Samples were collected at 3, 6, 9 and 12 hours post waking. It was imperative that volunteers abstained from food and drink (other than water) for a minimum of 30minutes prior to collecting the sample (to prevent food debris and foreign liquids being absorbed by the cotton wool).

#### **4.3.5 Data Treatment & Analysis**

All data were analysed using SPSS for Windows Version 12 (SPSS, Inc.) Cortisol data shows a general tendency for positive skewness (e.g. Edwards et al., 2001). The cortisol data was explored and normality assessed visually using histograms and statistically using the Kolmogorov-Smirnov test of normality. The cortisol data was positively skewed across all sample points and was normalised using a logarithmic transformation.

Diurnal profiles were constructed for each of the three days of sampling for each individual to observe the inter- and intra-subject consistency of the basal salivary cortisol profile. These profiles were used to calculate a number of indices of cortisol activity (previously described in Section 3.3.3) which were used to compare profiles using a correlational analysis. The indices were tested for consistency using a Pearson's Correlational analysis for each cortisol index across the three days for the 8 volunteers. This assessed the consistency of cortisol activity over the 3 days and the feasibility of the construction of an aggregate profile could be considered. This pilot study included assessment of a small and fairly diverse sample in terms of age and gender, therefore the analysis performed was an idiographic, graphical data analysis of the profiles with reference to the demographical and psychological characteristics of each volunteer.

The subject characteristics of the sample were compared using corrected t-tests to control for violations of homogeneity of variance concurrent with small sample sizes.

## 4.4 Results

### 4.4.1 Sample Characteristics

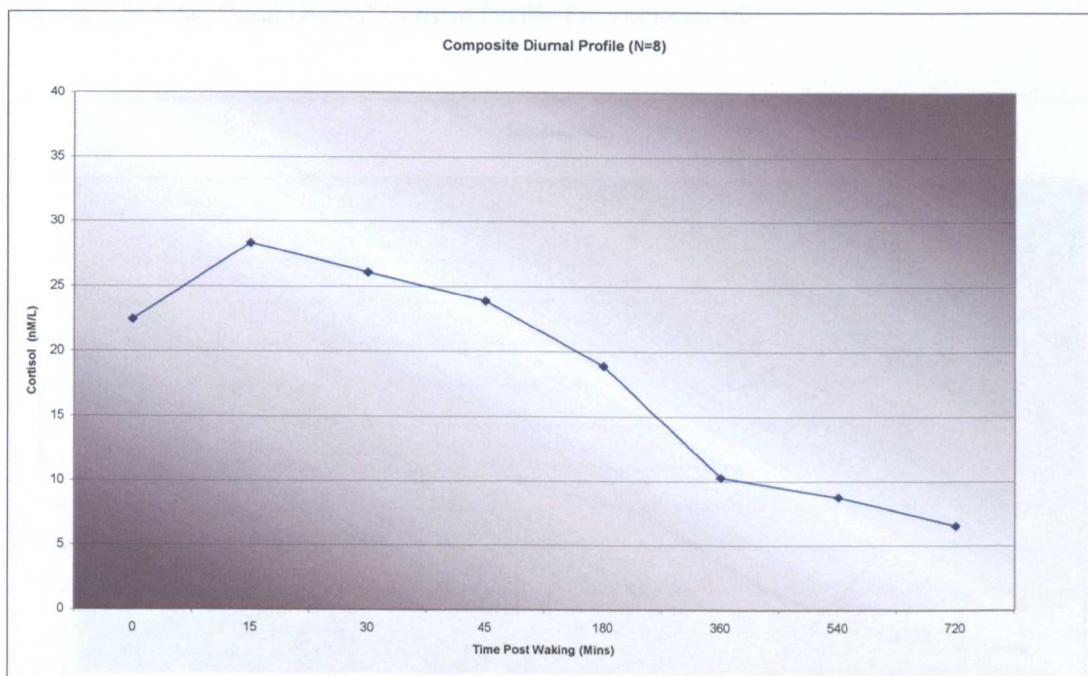
The characteristics of the sample are shown in Table 4.1 below.

Characteristic	Total N = 8	Male N = 3	Female N = 5
Age (years)	50.25 ± 6.11	53 ± 4.58	48.60 ± 6.77
BMI (Kg/m <sup>2</sup> )	27.74 ± 4.42	27.50 ± 3.54	27.84 ± 5.11
WHR	0.85 ± 0.09	0.96 ± 0.04	0.81 ± 0.06
PSS Score	17.88 ± 5.84	19.67 ± 7.10	16.80 ± 5.54

**Table 4.1:** Sample Characteristics displaying age, BMI, WHR & Perceived Stress with Means ±SD

There were no differences between males and females in terms of age, BMI or PSS score. Measured waist-hip ratio was significantly greater in males compared with females ( $t(5) = -3.021$ ;  $p = 0.023$ ).

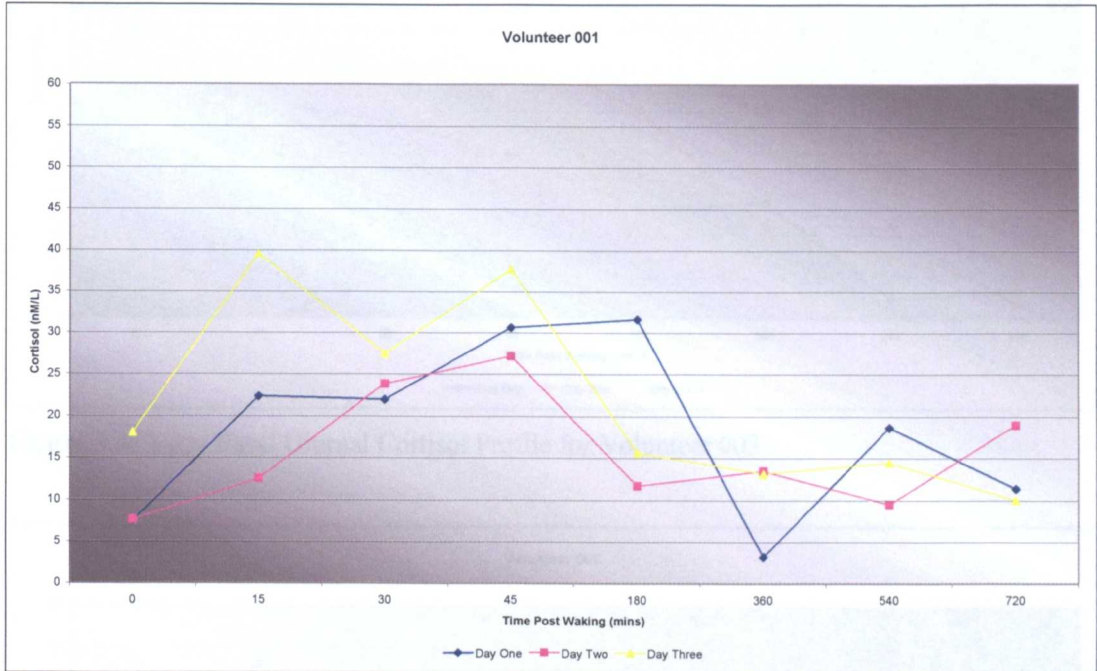
### 4.4.2 Diurnal Profiles



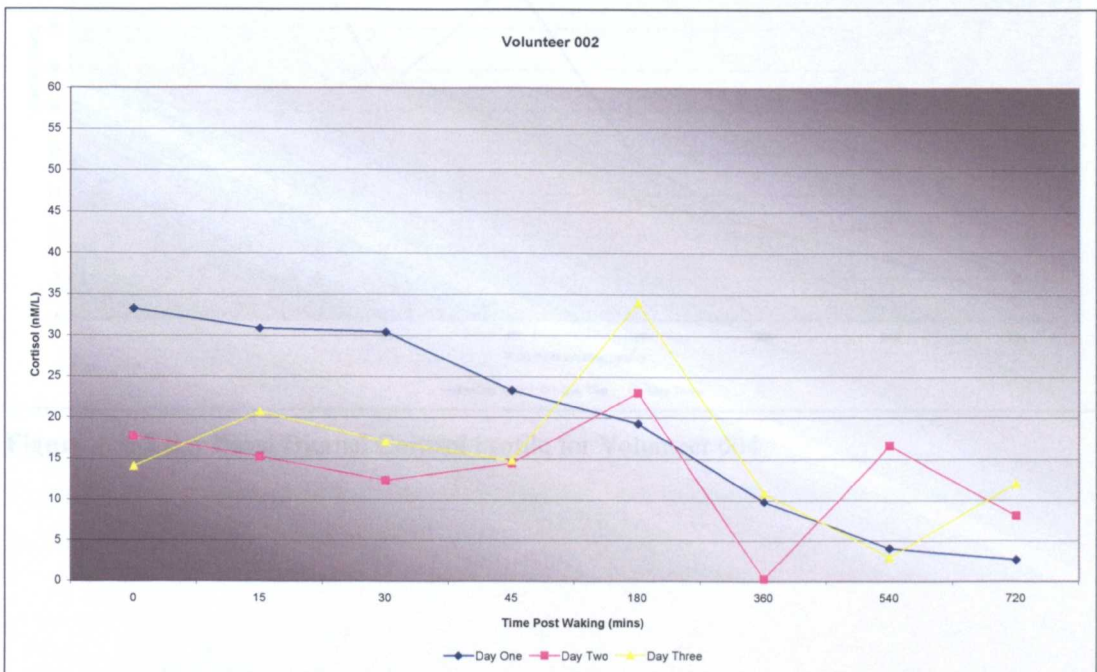
**Figure 4.1:** Composite Diurnal Cortisol Profile for the sample (Means ± SEM)

Figure 4.1: 3 day Diurnal Cortisol Profile for Veterans Mitz

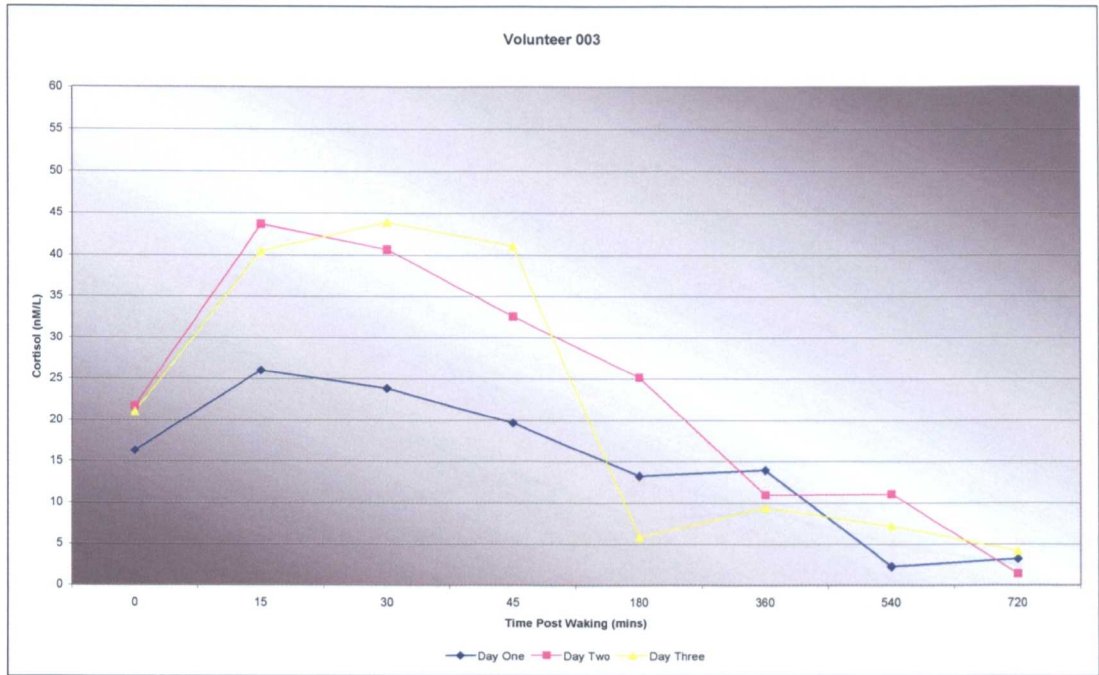
A composite profile based on the average of all the profiles for each volunteer in the sample was constructed (Figure 4.1). A set of diurnal profiles for each of the 3 days were constructed for each volunteer and are shown with their related psychological and physiological characteristics (Figure 4.2 - 4.9 and Table 4.2 - 4.5).



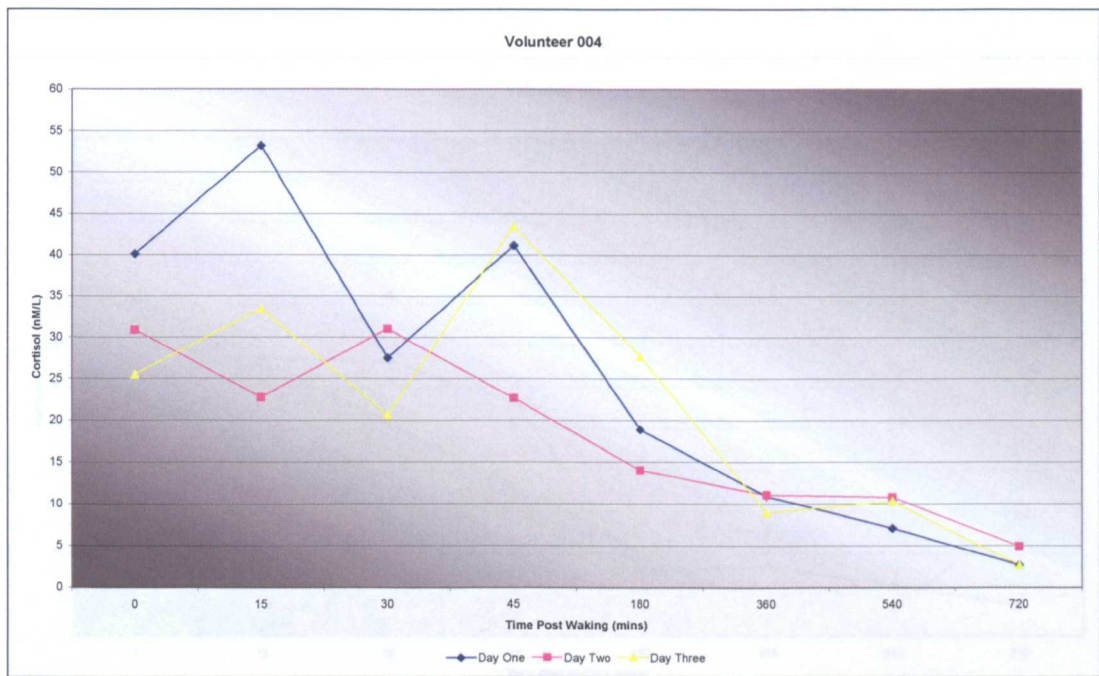
**Figure 4.2:** 3 day Basal Diurnal Cortisol Profile for Volunteer 001



**Figure 4.3:** 3 day Basal Diurnal Cortisol Profile for Volunteer 002

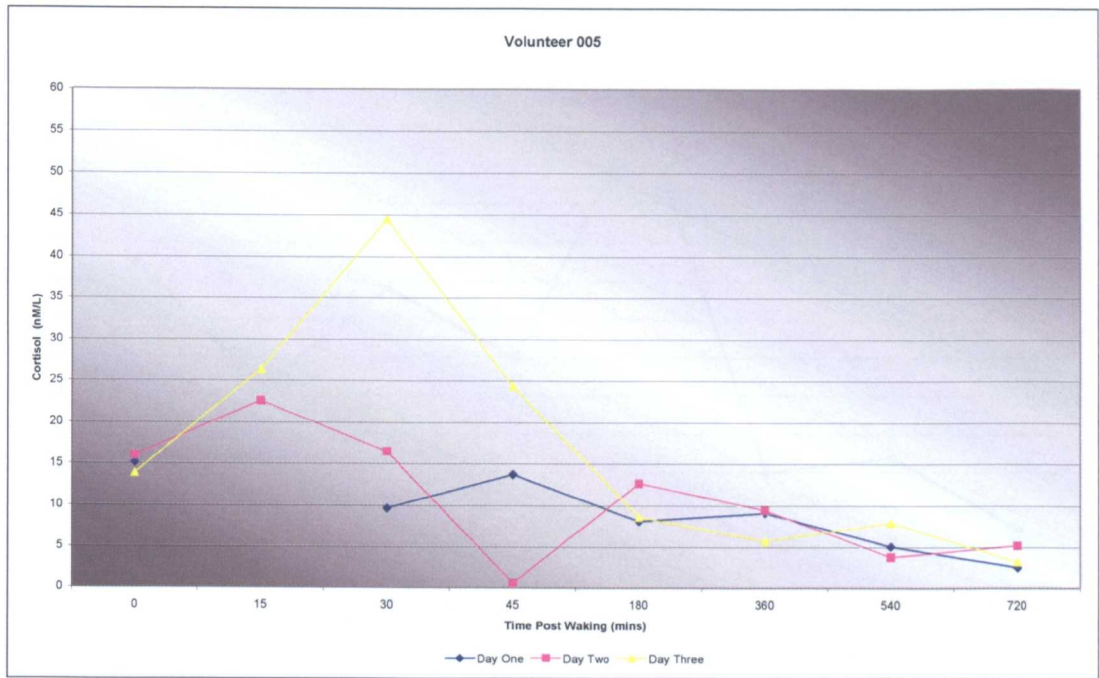


**Figure 4.4:** 3 day Basal Diurnal Cortisol Profile for Volunteer 003

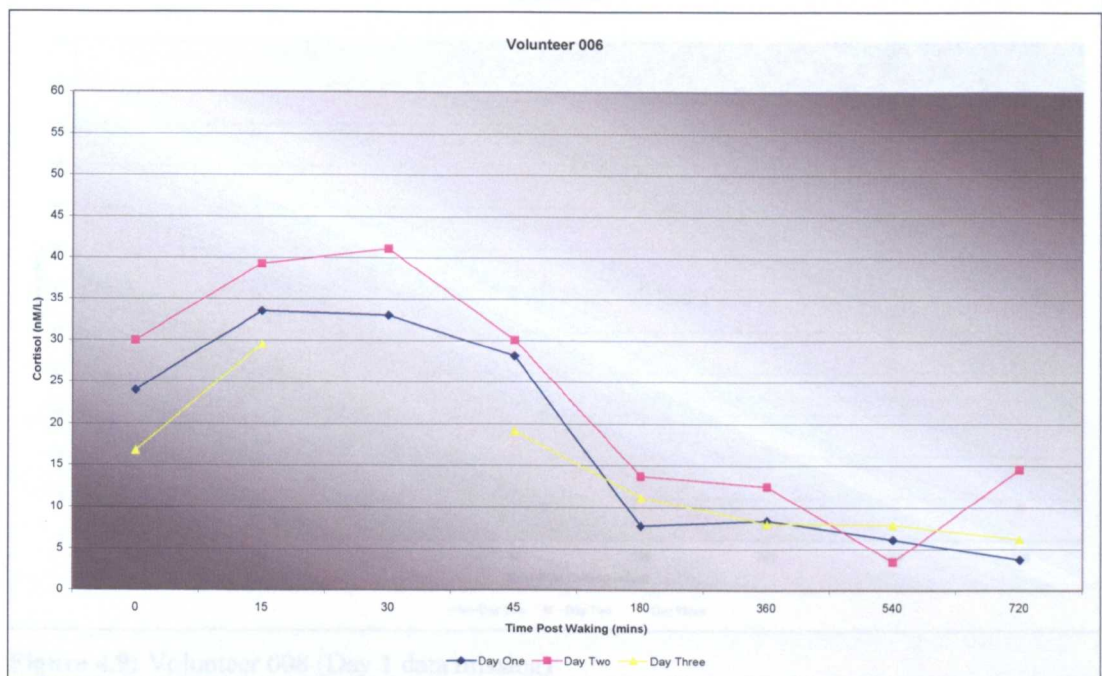


**Figure 4.5:** 3 day Basal Diurnal Cortisol Profile for Volunteer 004

**Figure 4.7:** 3 day Basal Diurnal Cortisol Profile for Volunteer 006 (missing data at these 30mins on Day 3)

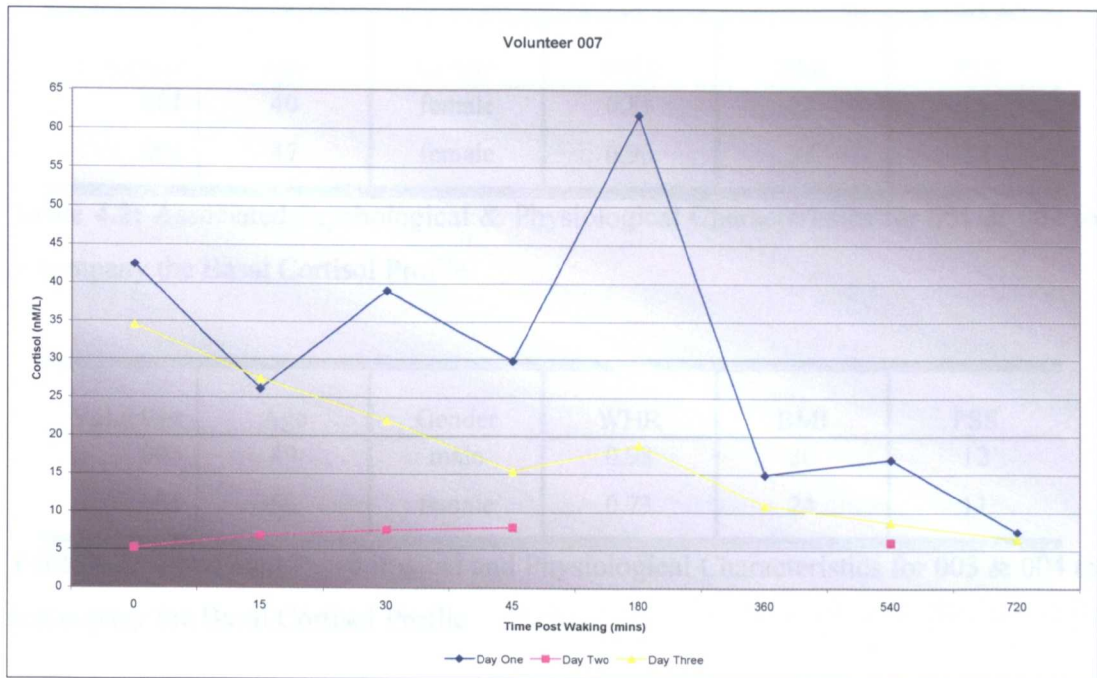


**Figure 4.6:** 3 day Basal Diurnal Cortisol Profile for Volunteer 005 (missing data at times 0 & 15mins on Day 1 & 45mins on Day2)

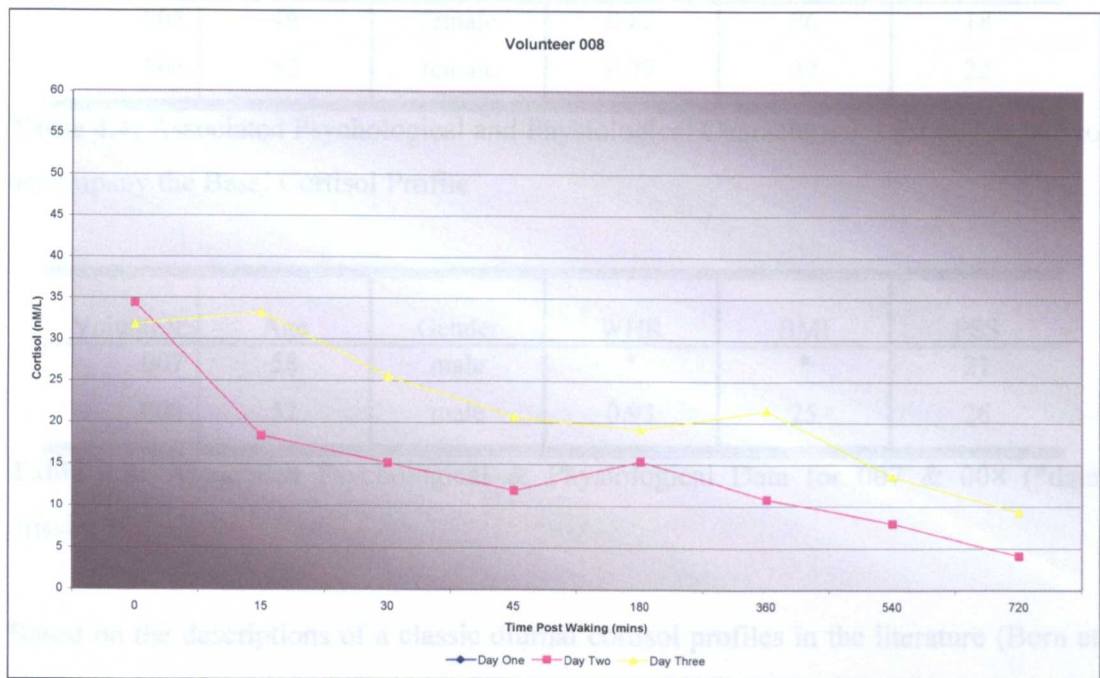


**Figure 4.7:** 3 day Basal Diurnal Cortisol Profile for Volunteer 006 (missing data at time 30mins on Day 3)





**Figure 4.8:** Volunteer 007 (Day 2 data missing)



**Figure 4.9:** Volunteer 008 (Day 1 data missing)

Volunteer	Age	Gender	WHR	BMI	PSS
001	40	female	0.81	23	11
002	47	female	0.90	35	22

**Table 4.2:** Associated Psychological & Physiological Characteristics for 001 & 002 to accompany the Basal Cortisol Profile

Volunteer	Age	Gender	WHR	BMI	PSS
003	49	male	0.98	30	12
004	58	female	0.73	24	11

**Table 4.3:** Associated Psychological and Physiological Characteristics for 003 & 004 to accompany the Basal Cortisol Profile

Volunteer	Age	Gender	WHR	BMI	PSS
005	46	female	0.82	26	18
006	52	female	0.77	32	22

**Table 4.4:** Associated Psychological and Physiological Characteristics for 005 & 006 to accompany the Basal Cortisol Profile

Volunteer	Age	Gender	WHR	BMI	PSS
007	58	male	*	*	21
008	52	male	0.93	25	26

**Table 4.5:** Associated Psychological & Physiological Data for 007 & 008 (\*data missing)

Based on the descriptions of a classic diurnal cortisol profiles in the literature (Born et al., 1999; Hucklebridge et al., 1998; Prüssner et al., 1997), it is evident that volunteer's 003 (Figure 4.4) and 006 (Figure 4.7) show typical profiles on each of the 3 sampling days. This suggests that it is possible to reproduce the basal diurnal cortisol profile using the methods adopted in this pilot for some individuals. What is interesting is the amount of variation even in this small sample. Volunteer 001 (Figure 4.2) demonstrates a good cortisol awakening response but demonstrates disrupted activity over the subsequent diurnal period. Volunteers 002 (Figure 4.3) and 004 (Figure 4.5)

demonstrated a flattened awakening response (no increase after the initial sample) but demonstrated a steady decrease in cortisol over the diurnal period. This was consistent across the 3 sampling days for these volunteers.

The profiles obtained for volunteers 007 (Figure 4.8) and 008 (Figure 4.9) were incomplete due to inadequate volumes of saliva collected. The profiles obtained were flattened, showing great variability, lacking a cortisol awakening response and remaining elevated over the remaining diurnal period. Volunteer 005 (Figure 4.6) also showed flattened profiles on 2 of the 3 days sampled. Day 3 of sampling for volunteer 005 produced a classic basal profile which suggests that there is some variability in the circadian rhythm of cortisol over the 3 days for this volunteer. This may indicate an improvement in compliance as the volunteer became familiar with the sampling protocol.

Minimal psychological assessment was made and as a result, it cannot be ascertained what the variability may be attributable to. For example, a greater experience of daily hassles or daily stressors may produce a more variable profile. In the current study, minimal associations with subjective stress (using the PSS) were noted. Volunteer 002 reported high perceived stress, concurrent with a highly variable profile, however this was not consistent for all volunteers. It is interesting to note high perceived stress in volunteers 007 and 008 who also suffered the greatest loss in cortisol data.

All the indices of cortisol activity (as described in Chapter Three, Section 3.3.3) were calculated (Day Mean, Diurnal Mean, AUC, AURC, MnInc, Day Difference, Waking Difference, Day Difference 3-12, Final Sample) for each profile.

Cortisol Index	D1-D2 N=6	D1-D3 N=7	D2-D3 N=6
Mean Increase	r = 0.783*	r = 0.751	r = 0.108
AUC	r = -0.128	r = -0.444	r = 0.541
AURC	r = 0.829*	r = 0.721	r = 0.138
Change 0-30	r = 0.852*	r = 0.098	r = 0.302
Day Mean	r = 0.361	r = 0.776*	r = 0.344
Diurnal Mean	r = 0.673	r = 0.683*	r = 0.279
Day Difference 0-12	r = 0.668	r = 0.344	r = 0.639
Day Difference 3-12	r = 0.460	r = 0.599	r = -0.014
Final Sample	r = 0.579	r = 0.510	r = 0.469

**Table 4.6:** Correlations for consistency across 3 sampling days for each cortisol index (\*p<0.05; \*\*p<0.01)

The profiles correlated well over the initial 2 days of sampling (Table 4.6) for mean increase, AUC and the difference in cortisol between waking and 30minutes post waking (Change 0-30). The mean of cortisol (both Day mean and Diurnal mean) correlated well over a longer period of time (D1-D3). The consistency of the Final Sample index, although not significant, did show moderate consistency across the three days. There was no evidence of more consistency between days 1 and 2 than for days 1 and 3 or 2 and 3 to indicate consistency over a longer period of time. These observations coincide with the degree of variability observed in the profiles both within individuals for each of the three days and between individuals in terms of level and pattern of activity (Figures 4.2-4.9).

#### 4.5 Discussion

This study was designed to assess the feasibility and practicality of using 3 day diurnal salivary cortisol profiles in a free living environment. Further, the feasibility of producing an aggregate profile from three separate sampling days was explored. Due to the small sample size and minimal psychological assessment in conjunction with basic cortisol assessment, inferences made are by necessity, tentative. However, a number of points for discussion can be raised.

The profiles presented in this study demonstrated a degree of inter- and intra subject variability in terms of the profile produced. It is clear that for some individuals, profiles are reproducible over a 3 day period, but it is also clear that deviations from a classic 'textbook' profile are common for a number of volunteers. It is of importance to highlight that although not all volunteers displayed a classic diurnal profile, the profiles that were exhibited were consistent across the 3 days of sampling.

The study also highlighted a number of practical and methodological issues that must be considered when using saliva samples for cortisol analysis.

#### **4.5.1 Inter- & Intra- Subject Variability**

The cortisol diurnal profile can demonstrate great both inter- and intra- variability in both the cortisol awakening response and subsequent diurnal activity. This conflicts with previous research which suggests moderate intra-individual stability using similar correlational analyses (Edwards et al., 2001; Wüst et al., 2000). There is great variation in the shape of the profile and in the consistency of the profile presented across the three days. The indices of cortisol activity used to compare level of cortisol activity between individual were based on an aggregated cortisol profile from 3 days of saliva sampling in a procedure that is similar to previous studies (e.g. Edwards et al., 2001). It could be misleading to compare individuals on their aggregate cortisol activity when it may be that the underlying profiles themselves are highly inconsistent. Equally, if only one day had been sampled, one would have been unaware of this and would have assumed that the one day was typical of the basal cortisol profile for that individual. Previous research has highlighted the importance of verifying the consistency of the profile prior to forming the aggregate profile (Edwards et al., 2001) and the current study reinforces the importance of this.

The findings of this study highlight the need to consider potential variation in a cortisol profile prior to its implementation in comparative research. Of the eight sets of profiles obtained, only two individuals (003 and 006) exhibited a classic cortisol awakening response (CAR). This evidences the potential to reproduce the cortisol diurnal profile, and particularly the cortisol awakening response using this procedure. However, the observation of a cortisol awakening response in only two out of eight data sets suggests low prevalence of the response in the current sample. It is necessary to examine why the

profiles exhibited were not classic for the remaining volunteers. There is an obvious lack of psychological data, it is possible that some individuals experienced greater daily stressors which were reflected in the profiles. Based on the available data, no associations were found in terms of subjective stress using the Perceived Stress Scale and no other differences in age, BMI or WHR were noted. This is will be considered in Chapter Five.

#### **4.5.2 Methodological Issues**

Volunteer compliance is paramount when conducting free-living studies which rely upon volunteer adherence to a demanding protocol. A number of issues relating to compliance were raised in this study.

##### **(i) Accurate Sample Timing**

Sample times were individually synchronised to waking time in this pilot study. This is heavily reliant on the volunteer noting the time of waking and calculating subsequent sample times for the remainder of the sampling day. Cortisol responses to forced versus natural waking have been explored by Hucklebridge et al. (1999). Hucklebridge et al. (1999) concluded that the cortisol awakening response can be influenced by the time of waking. For example, forced waking four hours earlier than normal disrupts the awakening response compared with uninterrupted waking or natural nocturnal waking. Bailey and Heitkemper (1991) found that the time of waking can affect the awakening response. Edwards et al. (2001) also found that the time of waking can alter the pattern of cortisol activity over the diurnal period. Therefore in the subsequent studies conducted in this thesis, cortisol sampling was synchronised to natural waking time. Further differences in the cortisol awakening response have been observed between weekday and weekend cortisol measurement (Kunz-Ebrecht et al., 2004; Thorn et al., 2006). As the sampling in the current study was conducted on week days, it is not possible to explore this in the current data set. However, in the subsequent studies conducted in this thesis, cortisol sampling will continue to be conducted on week days to prevent the influence of a confounding factor.

Once the sample times have been determined, the next step is to ensure that volunteers are compliant in producing the required samples at the required times. Volunteers were briefed at the induction session prior to the pilot study to emphasise the need for

accuracy when collecting saliva samples. It is difficult, however, to guarantee adherence. Previous research has highlighted the importance of compliance in saliva sampling using the methods outlined in the current study and has suggested the implementation of a form of electronic monitoring device (Kudielka et al., 2003). However, such devices are not infallible and could potentially produce forced waking. In an attempt to preserve the integrity of the data, Edwards et al. (2001; 2000) have suggested that individuals not displaying a cortisol awakening response should be excluded from data analysis (i.e. a profile that does not show an increase in cortisol activity after the initial sample). This is based on the assumption that the awakening response is an inherent response in individuals and a lack of response could signal the non-compliance. In the current study, this would have meant that 8 out of the 24 profiles obtained would have been excluded from the analysis or that 4 out of 8 volunteers would have been removed. It is unlikely that 50% of the sample were non-compliant.

It is also of interest, not only from a methodological perspective, to examine the proportion of profiles that show a non-classic profile since previous research has suggested that abnormal profiles may be associated with poor health outcomes (Bjorntorp and Rosmond, 2000). Thus in the subsequent studies presented in this thesis, attention is paid to the prevalence of classic and non-classic profiles and the likelihood that these are due to non-compliance as opposed to other psychological or physiological factors.

#### **(ii) Sample volume**

The biochemical assay procedure applied to the saliva samples for cortisol determination relies upon the collection of an adequate volume of saliva. Volunteers 007 and 008 failed to provide adequate volumes for the 8 samples collected on day three and hence no profile could be obtained. Volunteers were asked at the induction session to hold the cotton wool insert in the mouth for a minimum of thirty seconds (ideally it should be retained in the mouth for one minute), to allow for complete saturation of the cotton wool with saliva. This is again reliant on volunteer adherence and compliance. The majority of missed samples occurred during the cortisol awakening response which implies that these samples are the most difficult to obtain possibly due to a lack of saliva. Many volunteers did report the tendency for a dry mouth upon waking and hence perceived it to be difficult to produce an adequate volume of saliva.

### **(iii) Movement during the Cortisol Awakening Response**

Postural adjustment can stimulate the HPA axis due to a shift in blood volume. Hennig et al. (2000) proposed that this can influence cortisol activity and consequently produce an elevated cortisol awakening response. However, in a recent assessment, Hucklebridge et al. (2002) found no influence of movement or body posture on the cortisol awakening response. In the current study, it was decided that movement be restricted as a precaution in light of conflicting literature. Movement was advised to be kept at a minimum (remaining in a supine position) until the sample 30minutes post waking has been provided. This protocol was adhered to in the subsequent studies conducted in this thesis.

## **4.6 Conclusions**

This pilot study has highlighted some important methodological issues and considered the procedures adopted by previous research to decide upon the practicalities of saliva sampling for cortisol determination. It has also been informative to assess the feasibility of measuring cortisol diurnal profiles in a free living environment. The study has shown that a great deal of variability exists both within and between subjects, which has important implications for how cortisol profiles are interpreted and compared across a number of variables and individuals. The data suggests that it is important to assess profile consistency before any assertions about basal cortisol activity can be made. It is also important to implement strict yet feasible procedures to maximise the accuracy of the data obtained and to consider how best to relay these instructions to the volunteers in subsequent studies. The pilot study presented in this chapter suggests that cortisol profiles can be obtained, that 3 days of sampling is useful to ascertain intra-subject variability and that demographic and psychological variables may be important in examining inter-subject variability. This strategy was adopted for the studies subsequently presented in this thesis.



---

---

**CHAPTER FIVE****Basal Cortisol variability in older adults: Exploring associated biopsychological parameters**

---

---

**5.1 Introduction**

Chapter One reviewed a wealth of literature which suggests that those individuals who have a large amount of central fat or central obesity also exhibit altered levels of basal cortisol activity. Some earlier studies, which did not consider the diurnal fluctuation in cortisol, suggested that basal levels of plasma cortisol are elevated in obese individuals (Simkin et al., 1961; Szenas and Pattee, 1959). Increased understanding of obesity and its morphology indicates that the type of fat distribution is an important determinant of basal cortisol activity. Basal cortisol activity is elevated in individuals who exhibit peripheral obesity (Rivera and Svec, 1989). For those individuals who exhibit central obesity the same does not hold true; basal cortisol activity is altered but detectable levels of cortisol are lower (Ljung et al., 1996; Marin et al., 1992; Strain et al., 1980).

The fatty tissue type and distribution differs in the centrally obese compared to those with peripheral obesity (see Chapter One, Section 1.5.1). Cortisol production in the centrally obese *is* elevated but this elevation is not readily detectable in the basal circadian profile. This is due to an enhanced cortisol clearance rate (Ljung et al., 1996; Marin et al., 1992; Strain et al., 1980) evidenced by an increase in cortisol metabolites in urine and an elevated cortisol activity over the nocturnal period. This cortisol hypersecretion has been suggested to produce a blunted cortisol awakening response (due to dysregulation of the hypothalamic-pituitary-adrenal axis, which lowers cortisol peaks and raises troughs in activity). For example, Duclos et al. (1999) found that salivary cortisol measurements taken over the diurnal period in centrally obese individuals were lower in concentration compared to non-centrally obese volunteers.

Thus, individuals with central obesity and those with peripheral obesity display basal cortisol profiles that differ from lean individuals. Central and peripheral obesity

produces profiles distinctly different from each other. However, research has not demonstrated this finding with great consistency (Andrew et al., 1998; Ljung et al., 2000; Phillips et al., 1998; Phillips et al., 2000; Rask et al., 2002; Van Cauter et al., 1996; Wallerius et al., 2004; Ward et al., 2003). Moreover, studies, which have examined cortisol profiles over the diurnal period, have tended to sample only one day and have varied in the timing and number of samples taken (Steptoe et al., 2004; Edwards et al., 2001). Hence, the profile of inter and intra daily variation among lean, central and peripheral obese individuals requires confirmation.

From the detailed discussion of the metabolic syndrome (MS) in Section 1.5, it was evident that some of the biological parameters associated with metabolic syndrome were also common in central obesity. For example, individuals with central obesity often exhibit impaired glucose tolerance and/or insulin resistance (Rivera and Svec, 1989; Hartz et al., 1984), known components of the Metabolic Syndrome. Other common characteristics include hypertension and elevated blood lipids. The observation that central obesity and cortisol dysregulation co-occur, for example in patients with Cushing's syndrome (Starkman et al., 1999; 2003), suggested a possible role for cortisol in the expression of the metabolic syndrome. Bjorntorp and Rosmond (1999; 2000; 2001) labelled metabolic syndrome as a neuroendocrine disorder of cortisol dysregulation based on stress released cortisol. Although studies have not examined basal diurnal cortisol profiles in MS it has been suggested that cortisol is altered in this condition. Key metabolic symptomology are associated with altered glucocorticoid activity and hypothalamic-pituitary-adrenal axis regulation (Andrew et al., 2002; Bjorntorp and Rosmond 1999; 2000; 2001; Rosmond et al., 2000). Since individuals with central obesity may develop Metabolic Syndrome or may already be manifesting undiagnosed symptoms, the identification of altered cortisol profile and the association of this with other parameters of the metabolic syndrome may be of theoretical and practical importance.

## **5.2 Objectives**

This study is an examination of the consistency and replicability of basal salivary cortisol activity in males and females aged between 35 and 65 years. The study examines whether differences in basal glucocorticoid activity can be related to

abdominal adiposity specifically waist-hip ratio. The study also examines the relationship between salivary cortisol activity and biological parameters that are associated with metabolic syndrome, for instance, glucose, insulin and blood lipids (Bjorntorp 1997) and inflammatory markers, which reflect immune system activity.

### **5.3 Method**

#### **5.3.1 Sample**

Volunteers were recruited through a variety of sources. Small adverts were inserted in University Magazine 'The Reporter', which is distributed widely throughout the University of Leeds campus. This document is also available as an 'online magazine' readily accessible to university members at any time both on and off campus. Posters were distributed about the university precinct, particularly in the students union and in various departmental staff/canteen rooms at the discretion of departmental secretaries. Posters were also displayed in public libraries, gymnasiums and community centres around Leeds. Campus wide distribution lists were targeted using email fliers. A small advert was also placed in the local government recruitment/news bulletin.

One hundred and ten volunteers were initially recruited using opportunity sampling on a volunteer basis (58 females, 52 males). The exclusion criteria for the study are outlined below.

#### **Exclusion Criteria**

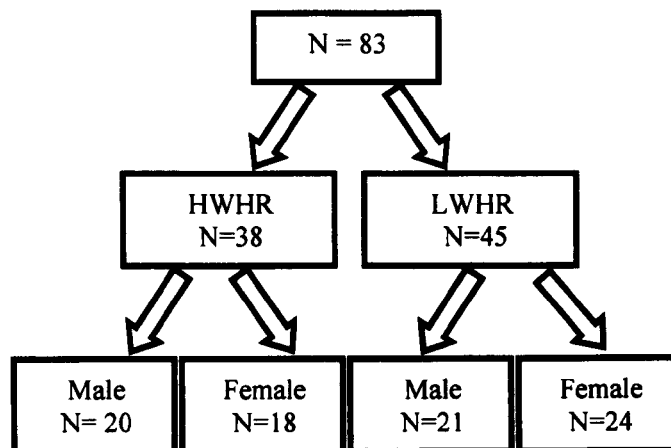
- Any person not within the specified age boundary (<35 or >65)
- Smokers or ex-smokers of less than one year.
- Night shift or abnormal shift workers
- Those who engage in excessive amounts of exercise regularly
- Any female on any form of Hormone Replacement Therapy currently or in the last 12months.
- Any person on any type of steroidal / anti – inflammatory / hormonal medication or any prescription or over the counter drugs in the past month, including recreational drugs
- Any individual using medication for psychological problems e.g. depression, anxiety etc. Similarly, anyone with a history of mental disorders /

psychological problems

- Anyone with a history of cardiac, neurological, hepatic, digestive, thyroid, renal or hormonal disorders etc, diabetes or asthma etc.
- Anyone with abnormal blood pressure and cardiac output (over 140/90mmHg at rest (WHO, 2000)).
- Anyone who has donated blood in the past month
- Anyone with a high score on the Hospital Anxiety and Depression Scale (HADS) indicating clinical caseness
- Anyone with a high score on the Dutch Eating Behaviour Questionnaire (DEBQ)

The final sample size for analysis was 83; 41 male and 42 females (Figure 5.1). All were aged between 35 and 65 with a mean age of 45.71 ( $\pm$  7.21 SD) years. Volunteers were not currently on any form of medication and were non-smokers. Ninety-three percent of the volunteers in the sample were in current full or part time employment (4% classed themselves as students, 2% as homemakers and 1% were retired).

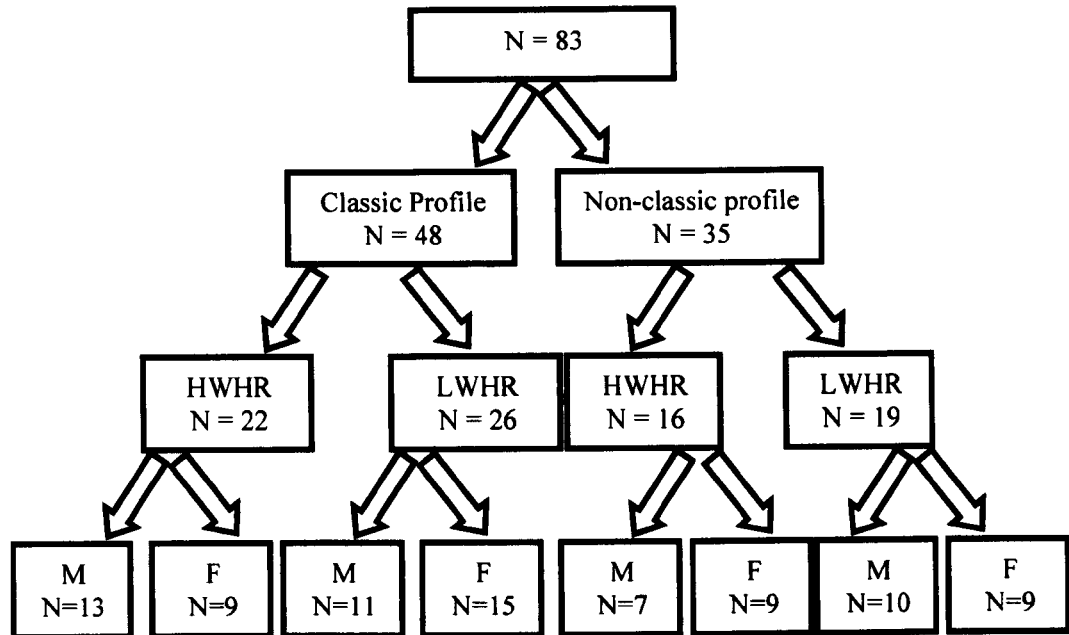
### 5.3.2 Design



**Figure 5.1:** Flow Chart to illustrate the main study design with WHR & gender as between subjects factors

The study conformed to a 2x2 ANOVA design (See Figure 5.1), with 2 between subjects' factors, waist-hip ratio and gender, each with 2 levels (high/low and male/female). For cortisol sampling, time was included as a within subjects variable in the ANOVA model with 8 levels.

When the characteristics of the profile were considered, an additional IV was introduced. The design thus conformed to a 2x2x2 ANOVA (Classic Versus Non-Classic) with 3 between subjects factors, profile group, waist-hip ratio group and gender, each with 2 levels (classic/non-classic, high/low and male/female) (See Figure 5.2).



**Figure 5.2:** Flow Chart to illustrate the Classic versus Non-Classic study design with WHR, gender & Profile Group as between subjects' factors

### 5.3.3 Measures

#### 5.3.3.1 Psychological Measures

The following measures were administered during the study (See also Chapter 3: General Methodology): (i) Perceived Stress Scale (PSS; Cohen, 1994). This was administered at screening and used to measure of the perception of stress and the degree to which situations in an individual's life are appraised as being stressful (see Appendix 10). (ii) Daily Hassles Scale (Kanner et al., 1981). This was used to assess the quality of the day in terms of the frequency and intensity of potentially stressful events/hassles encountered. The scale includes one hundred and seventeen items, which measure the frequency and severity of a person's transactions with the environment that are considered by the person to be stressful. The scale was amended to include a self-report section for the volunteer to detail any particular events occurring that were perceived as being particularly stressful on each of the three sampling days (see Appendix 15). In addition the following measures were administered:

**(i) Hospital Anxiety & Depression Scale (HADS; Snaith and Zigmond, 1994)**

The HADS is a widely used screening tool, implemented as an indicator of mood pathology, measuring subjective feelings of depression and anxiety (see Appendix 12). Scores of 0-7 in each of the two subscales are considered normal, scores of 8-10 are borderline and scores of 11+ are indicative of clinical 'caseness'. There is a possible third cut-off of 14/15 for 'severe' cases. The HADS is easy to administer and is quick to complete and to score. The HADS has demonstrated high acceptance ratings in both patients and individuals devoid of psychopathology (Herrmann et al., 1991). Assessment of the reliability of both subscales within the HADS yields satisfactory internal consistency (Cronbach alpha's) of 0.80 to 0.93 for the anxiety subscale and 0.81 to 0.90 for depression (Herrmann, 1997; Herrmann et al., 1995). Retest reliability correlates highly after up to two weeks demonstrating the stability of the scale over time and in response to situational influences (Elliott, 1993; Prettyman et al., 1993; Salkovskis et al., 1990). This measure was implemented as a screening tool and was not subsequently analysed in the results.

**(ii) Dutch Eating Behaviour Questionnaire (DEBQ; Van Strien et al., 1986)**

The DEBQ measures three facets of eating behaviour; (i) restraint (ii) emotional eating and (iii) external eating (see Appendix 13). Responses are formed on a scale of 1-5 with an average score for each subscale. The DEBQ collectively measures these facets in one easily administered questionnaire. Studies since its development the DEBQ has been found to have high internal consistency ( $\alpha = .95$ ) and good two week test re-test reliability ( $r = .82$ ) (Stice, 2001; Van Strien et al., 1986). This measure was implemented as a screening tool and was not subsequently analysed in the results.

**(iii) Leeds Sleep Evaluation Questionnaire (LSEQ, Parrott and Hindmarch, 1980)**

The LSEQ assesses four aspects of sleep/sleeping behaviour: (i) getting to sleep (ii) quality of sleep (iii) awakening from sleep and (iv) behaviour following waking. The questions tap into subjective opinion of aspects of sleep and early morning activity (see Appendix 11). Responses are made using a visual analogue scale (1-100). Compared with other measures of sleep, one advantage of the LSEQ is that it contains more questions (ten). Therefore, is able to provide a more detailed assessment of sleep compared to scales applied by Bond and Lader (1974) and Nicholson et al. (1976). The use of visual analogue scales for subjective ratings is a widely used and validated

approach (Aitken, 1969; Herbert et al., 1976). EEG studies demonstrate that visual analogue measures of sleep correlate well with objective EEG recordings (Lewis, 1969). The LSEQ has good retest reliability (0.63-0.78, Tarrasch et al., 2003) and stability across a range of clinical settings (Zisapel and Laudon, 2003).

### **5.3.3.2 Biological Measures**

The following physiological measures were obtained at screening to assess body shape and current health status. These included; (i) blood pressure, (ii) body mass index (BMI), (iii) body composition (fasted), (iv) waist-hip ratio (WHR) and (v) a range of blood biomarkers including fasting insulin and glucose, total cholesterol, HDL, LDL and triglycerides, IL-6, CRP and adiponectin (see Chapter Three Section 3.2.3.1)

Salivary cortisol was measured frequently over the course of the study using pre-labelled salivettes (Sarstedt). Eight salivettes were provided for each volunteer per day sampled labelled in accordance with the sample day and time. Three sampling days were undertaken providing twenty-four salivettes per volunteer in total. The data collected was used to construct a cortisol diurnal profile for each day.

Dehydroepiandrosterone (DHEA) was assessed on each of the three test days over the course of the study. One passive drool sample using a small pre-labelled collection pot was collected per day.

### **5.3.4 Procedure**

Potential volunteers were screened via telephone interview (see Appendix 2). Suitable volunteers were sent a Volunteer Screening Booklet (see Appendix 1). Selected volunteers were asked to attend an induction session at the Institute of Psychological Sciences. This session was designed to introduce the volunteer to the study, the equipment and procedure. The study was approved by the Institute of Psychological Sciences Ethics Committee prior to the study commencement.

#### **5.3.4.1 Cortisol Monitoring Period**

Volunteers were asked to refrain from the consumption of alcohol on the nights prior to the experiment since alcohol can elevate cortisol levels on the subsequent sampling day. The following protocol was thus adhered to (see Appendix 3):

- i. An initial sample for DHEA assessment was produced immediately upon waking using the drool pot provided. This was conducted whilst the volunteer was still in bed, in a supine position. No food or drink other than water (if necessary) was permitted at this time.
- ii. **Immediately** following this, the volunteer was asked to produce a saliva sample for cortisol assessment using the designated salivette provided. The volunteer was asked to remain in a supine, relaxed position and to abstain from food and drink.
- iii. The next sample was taken 15 minutes post waking as in step (ii) using the designated salivette provided.
- iv. The same procedure was repeated at 30 minutes and 45 minutes post waking. The volunteer was asked to remain in a supine position until after the 30-minute sample. Volunteers were asked to refrain from consuming any food or drink other than water until after the 45-minute sample. Additionally, volunteers were asked to refrain from brushing teeth until this sample had been collected (to avoid vascular leakage and micro-abrasion, which has been shown to alter the saliva in the sample – See Chapter Three). Subsequently, volunteers were asked to complete the Leeds Sleep Evaluation Questionnaire (LSEQ) to assess sleep quality during the previous night.
- v. Volunteers were asked to continue collecting saliva samples over the course of the afternoon. Samples were collected at 3, 6, 9 and 12 hours post waking. It was imperative that volunteers abstained from food and drink (other than water) for a minimum of 30minutes prior to collecting the sample (to prevent food debris and foreign liquids being absorbed by the cotton wool).
- vi. Before the volunteer retired to bed, volunteers were asked to complete the Daily Hassles Scale to evaluate opinion of the day in terms of the frequency and number of hassles/daily stressors encountered.

This procedure was repeated for three consecutive working/week days.



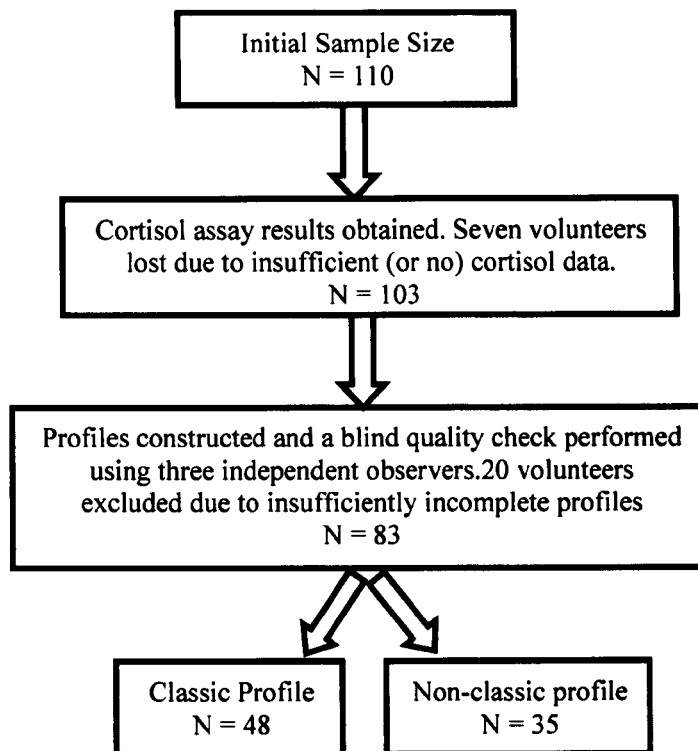
### **5.3.5 Data Treatment & Analysis**

#### **5.3.5.1 Missing Data**

Three independent observers (blind to waist-hip ratio or gender) considered each set of profiles. The profiles were characterised as 'classic' or 'non-classic' in shape. A classic profile was characterised by a clear response to waking followed by a steady decline to stable concentrations in the later diurnal stages. A non-classic profile was flattened, and lacked a cortisol awakening response with a less stable diurnal decline.

In the construction of the cortisol diurnal profile, data inclusion was based on each volunteer presenting at least two sampling days of cortisol data for each time point in order to aggregate the profile accurately. If this criterion was not satisfied then the volunteer was excluded from analysis. Interpolation of data points was possible (if and when necessary) during the diurnal period of the profile. However, no interpolations were permitted for awakening response data as cortisol is subject to less stable changes in concentration during the cortisol awakening response.

This research was dependent on volunteer compliance to produce the required samples at the specified times and to produce adequate volumes of saliva for accurate assay. As a result, the final analysis of cortisol did not include the full sample (n=110). The final sample size comprised 83 individuals with some variation for specific analyses (sample sizes are specified within the analysis of each hypothesis). The data for biomarker assessment was almost complete with 7 exclusions due to unsuccessful venepuncture. Figure 5.3 details the stages of volunteer exclusion and retention.



**Figure 5.3:** Flow chart to detail the stages of volunteer exclusion & retention

### 5.3.5.2 Data Screening

All data was screened for outliers using boxplots prior to analysis. The analysis was subsequently conducted with outliers included unless they were extreme outliers which had a major effect on the data, in which case the analysis was re-run with the outliers excluded. The cortisol data was explored and normality assessed using histograms and the Kolmogorov-Smirnov test of normality. The data was found to be positively skewed across all sample points and was subsequently normalised using a logarithmic transformation. All analyses were conducted using SPSS version 12.

As in Chapter 4, a correlational analysis for each cortisol index across the three days was conducted. This assessed the consistency of cortisol activity across the 3 days and the feasibility of the construction of an aggregate profile was explored prior to analysis.

### 5.3.5.3 Statistical Analysis

#### (i) Basal Cortisol

A 2x2x8 repeated measures ANOVA with waist-hip ratio (high/low) and gender (male/female) as between subjects factors and time as a within subjects factor was

conducted to explore basal diurnal cortisol in relation to waist-hip ratio and gender. Age was included as a covariate. Any significant interactions were explored further by post hoc tests using Bonferroni corrected independent sample t-tests. The basal profile was analysed in terms of the cortisol awakening response and subsequent diurnal activity using the calculated indices of cortisol activity (outlined in Chapter Three, Section 3.3.3), i. area under the curve; ii. area under the curve with reference to the first sample; iii. mean increase; iv. change 0-30; v. day difference 0-12; vi. day difference 3-12; vii. day mean; and viii. diurnal mean. These were analysed using separate 2x2 ANOVA's with each index included as the dependent variable. Age was included as a covariate in each analysis. Any significant interactions were explored further by post hoc tests using Bonferroni corrected independent sample t-tests. Linear regression was used to assess strength of the covariates relationship with each dependent variable and beta values are reported.

#### **(ii) Classifying Diurnal Profiles**

Following blind classification of the basal diurnal profiles by three independent observers, a separate between subjects variable 'profile group' was formed. A 2x2x2 ANOVA with waist-hip ratio (high/low) and gender (male/female) as between subjects factors and age as a covariate in each analysis, was conducted for each profile group using the cortisol indices as dependent variables to explore differences between the classic and non-classic profile classifications.

Each biomarker was assessed as a dependent variable separately in 2x2x2 ANOVAs with waist-hip ratio (high/low), gender (male/female) as between subjects' factors for each profile group (classic/non-classic). Age was included as a covariate in each analysis. To assess the relationship between each biomarker and basal cortisol activity, the calculated mean cortisol from the basal diurnal profile (Day Mean) was also included as a covariate in addition to the area under the curve with reference to zero (AUC). If covariates were significant, a linear regression was performed to examine the nature of the relationship between the covariate and the dependent variable. Any significant interactions were explored further by post hoc tests using Bonferroni corrected independent sample t-tests.

Each item on the Leeds Sleep Evaluation Questionnaire was analysed using a multivariate analysis of variance (MANOVA) with each item included as dependent variables and profile group, waist-hip ratio and gender as between subjects' factors. Mean cortisol over the diurnal period (Day Mean) and two measures of the awakening response (AUC and AURC) were included as covariates in the analysis of the LSEQ items. Any significant interactions were explored further by post hoc tests using Bonferroni corrected independent sample t-tests. Individuals were consistent in the time of waking across the 3 sample days, therefore a median split on the mean waking time data from the 3 days of sampling was performed. Based on this, the sample was split into those who awoke earlier than the median (early risers) and those who rose later than the median waking time (late risers). A chi-squared test of independence (2x2) was conducted to explore the possible association between waking time group and profile group. The data was subsequently analysed using the same approach as in the analysis of basal cortisol.

Perceived stress score and scores for intensity and frequency of daily hassles were assessed in relation to waist-hip ratio, gender and profile group using a 2x2x2 ANOVA. Mean diurnal cortisol (Day Mean) was included as a covariate. Any significant interactions were explored further by post hoc tests using Bonferroni corrected independent sample t-tests.

## **5.4 Results**

### **5.4.1 Sample Characteristics**

The main characteristics of the sample are presented in Table 5.1. WHR and BMI differed significantly between the high and low waist-hip ratio groups ( $F(1, 79) = 149.870$ ;  $p < 0.01$  and  $F(1, 79) = 64.550$ ;  $p < 0.01$  respectively). But the waist-hip ratio and gender groups did not differ by age ( $F(1,79)=0.761$ ;  $p=0.386$ ; NS and  $F(1,79)=0.281$ ;  $p=0.598$ ; NS). Perceived stress score significantly differed by gender ( $F(1, 79) = 4.642$ ;  $p < 0.05$ ). Females demonstrated a significantly greater perceived stress score than males. Further, a trend for a waist-hip ratio group\*gender interaction was observed ( $F(1, 79) = 3.247$ ;  $p = 0.075$ ). High waist-hip ratio females scored significantly higher than high waist-hip ratio males ( $p = 0.016$ ). High and low WHR females did not significantly differ ( $p = 0.106$ ).

Characteristic	Total N=83	Male LWHR N = 21	Male HWHR N = 20	Female LWHR N = 24	Female HWHR N = 18
Age (years)	45.71±7.21	45.05±7.86	45.30±7.01	47.54±7.31	44.50±6.62
BMI (Kg/m <sup>2</sup> )	27±5.57	24±2.63	30±5.60	24±2.70	32±5.31
WHR	0.87±0.08	0.85±0.04	0.96±0.04	0.79±0.04	0.92±0.06
PSS Score	15±6.77	14.57±4.55	13.20±6.35	15.08±7.38	18.94±7.61

**Table 5.1:** Sample Characteristics displaying age, BMI, WHR & Perceived Stress with Means ± SD

#### 5.4.2 Consistency of the Basal Diurnal Profile

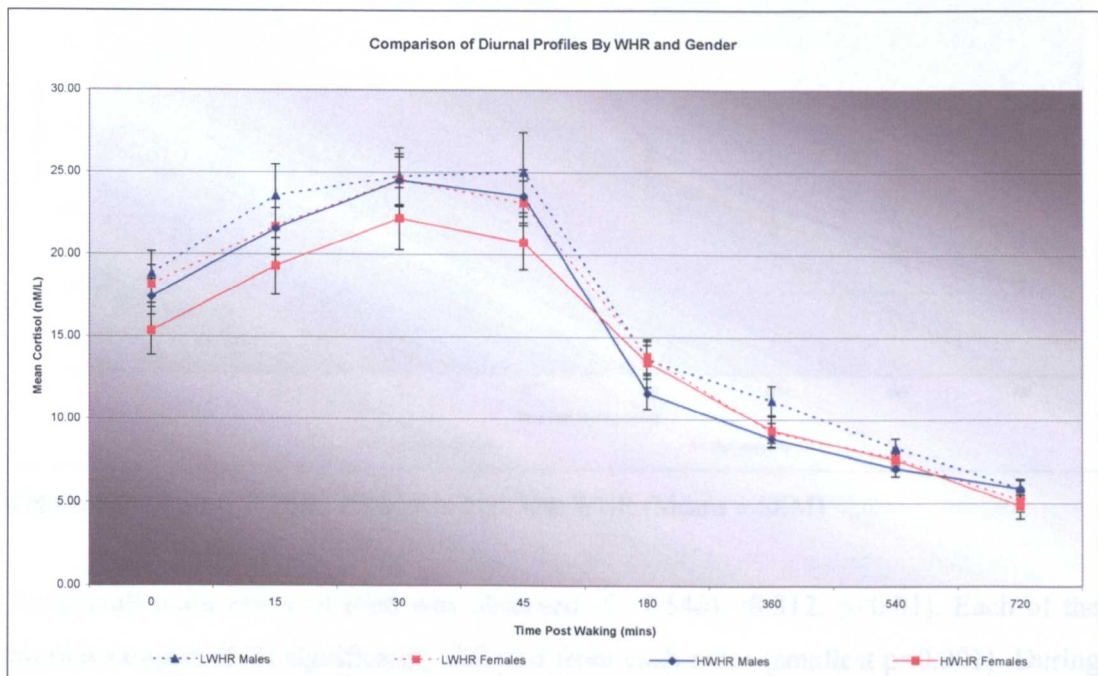
The profiles demonstrated good consistency across the three days of sampling as demonstrated in Table 5.2 below. In particular, area under the curve with reference to zero (AUC), the change in cortisol between waking and 30minutes post waking (Change 0-30), Day Mean, Diurnal Mean, Final Sample and the difference between waking and the Final Sample (Day Difference 0-12) were highly consistent across all three days of sampling. There was no evidence for a loss of consistency over a longer period of sampling. Despite demonstrating that some variation in the profiles is evident, this analysis largely supported the aggregation of the profiles for further analysis.

Cortisol Index	D1-D2 N=63	D1-D3 N=71	D2-D3 N=74
Mean Increase	r = 0.48	r = 0.228	r = 0.232
AUC	r = -0.673**	r = -0.673**	r = 0.637**
AURC	r = 0.107	r = 0.224	r = 0.255*
Change 0-30	r = 0.249*	r = 0.182	r = 0.268*
Day Mean	r = 0.719**	r = 0.512**	r = 0.634**
Diurnal Mean	r = 0.714**	r = 0.492**	r = 0.597**
Day Difference 0-12	r = 0.381**	r = 0.467**	r = 0.550**
Day Difference 3-12	r = 0.119	r = 0.125	r = 0.167
Final Sample	r = 0.199	r = 0.425**	r = 0.379**

**Table 5.2:** Correlations for consistency across 3 sampling days for each cortisol index (\*p<0.05; \*\* p<0.01)

### 5.4.3 Basal Cortisol in High/Low WHR Males & Females

The waist-hip ratio group\*gender interaction illustrated in Figure 5.4, failed to reach significance ( $F(1, 78) = 0.002$ ;  $p = 0.967$ ; NS).



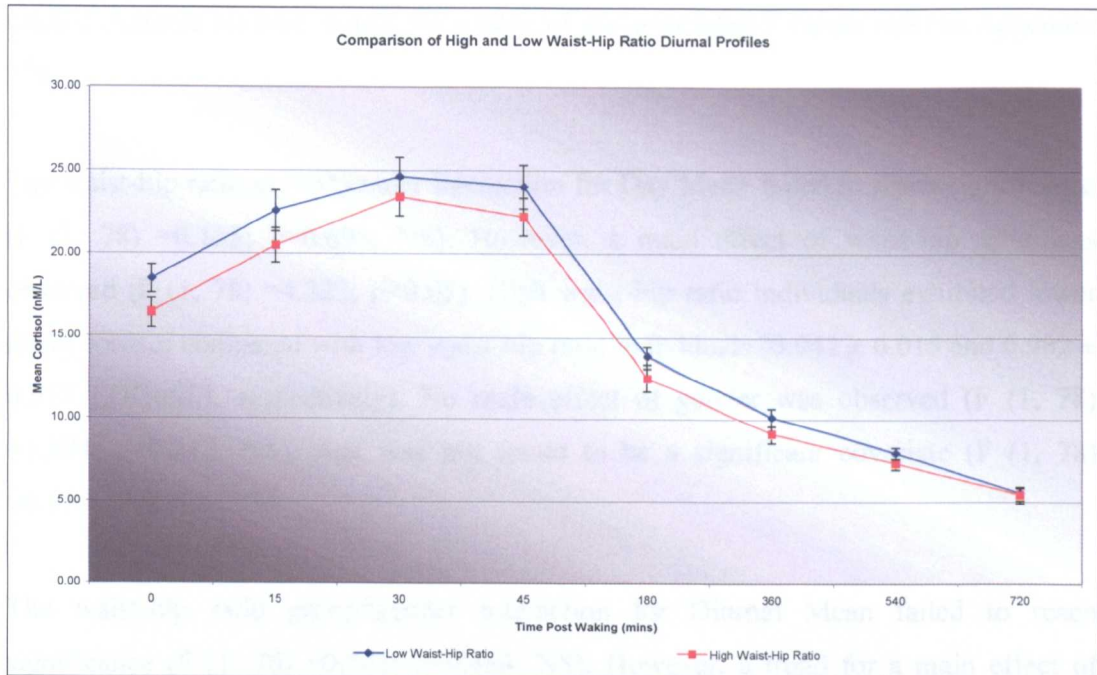
**Figure 5.4:** Basal Diurnal Cortisol Profiles in high/low WHR males/females (Means  $\pm$  SEM)

A trend for a main effect of waist-hip ratio group for mean cortisol secreted across the profile was observed (observed) ( $F(1, 78) = p = 0.063$ ). Figure 5.5 illustrates that high waist-hip ratio individuals exhibited lower mean cortisol compared with low waist-hip ratio individuals ( $1.084 \pm 0.015$  and  $1.123 \pm 0.014$  LOGnM/L respectively).

#### 5.4.3.1 Indices of Cortisol Activity in High/Low WHR Males & Females

The waist-hip ratio group\*gender interaction by Mean Increase failed to reach significance ( $F(1, 78) = 0.284$ ;  $p = 0.592$ ; NS). No main effect of waist-hip ratio ( $F(1, 78) = 1.612$ ;  $p = 0.208$ ; NS) or gender ( $F(1, 78) = 0.160$ ;  $p = 0.753$ ; NS) was observed. Age was not found to be a significant covariate ( $F(1, 78) = 0.046$ ;  $p = 0.831$ ; NS).

The same pattern of results was observed for the area under the curve (with reference to the first sample (AUC) and with reference to area (AUC), change from waking to 15 minutes post waking (Change 0-15), day difference from waking to final sample (Day Difference), day difference from sample 1 to sample 3 (Day Difference 3-12) and Final



**Figure 5.5:** Diurnal Cortisol Profiles in high/low WHR (Means  $\pm$  SEM)

An overall main effect of time was observed ( $F(7, 546) = 8.312$ ;  $p < 0.01$ ). Each of the diurnal samples (5-8) significantly differed from each other (smallest  $p = 0.001$ ). During the awakening response, sample 3 (30 minutes post waking) significantly differed from sample 1 (0 minutes) and sample 2 (15 minutes post waking). Sample 4 (45 minutes post waking) differed from sample 1 (0 minutes) but did not differ from samples 2 and 3.

No main effect of gender was observed ( $F(1, 78) = 2.583$ ;  $p = 0.112$ ; NS) and age was not found to be a significant covariate ( $F(1, 78) = 0.502$ ;  $p = 0.502$ ; NS).

#### 5.4.3.1 Indices of Cortisol Activity in High/Low WHR Males & Females

The waist-hip ratio group\*gender interaction for Mean Increase failed to reach significance ( $F(1, 78) = 0.289$ ;  $p = 0.592$ ; NS). No main effect of waist-hip ratio ( $F(1, 78) = 1.612$ ;  $p = 0.208$ ; NS) or gender ( $F(1, 78) = 0.100$ ;  $p = 0.753$ ; NS) was observed. Age was not found to be a significant covariate ( $F(1, 78) = 0.046$ ;  $p = 0.831$ ; NS).

The same pattern of results was observed for the area under the curve (with reference to the first sample (AURC) and with reference to zero (AUC)), change from waking to 30 minutes post waking (Change 0-30), day difference from waking to final sample (Day Difference), day difference from sample 5 to sample 8 (Day Difference 3-12) and Final

sample (Sample H) were noted (for a table of the associated F values refer to Appendix 17).

The waist-hip ratio group\*gender interaction for Day Mean failed to reach significance ( $F(1, 78) = 0.155$ ;  $p = 0.695$ ; NS). However, a main effect of waist-hip ratio was observed ( $F(1, 78) = 4.223$ ;  $p < 0.05$ ). High waist-hip ratio individuals exhibited lower mean cortisol compared with low waist-hip ratio individuals ( $0.942 \pm 0.015$  and  $0.982 \pm 0.014$  LOGnM/L respectively). No main effect of gender was observed ( $F(1, 78) = 1.584$ ;  $p = 0.212$ ; NS). Age was not found to be a significant covariate ( $F(1, 78) = 0.113$ ;  $p = 0.738$ ; NS).

The waist-hip ratio group\*gender interaction for Diurnal Mean failed to reach significance ( $F(1, 78) = 0.541$ ;  $p = 0.464$ ; NS). However, a trend for a main effect of waist-hip ratio was observed ( $F(1, 78) = 2.795$ ;  $p = 0.099$ ). High waist-hip ratio individuals exhibited lower mean cortisol compared with low waist-hip ratio individuals ( $0.881 \pm 0.017$  and  $0.920 \pm 0.016$  LOGnM/L respectively). No main effect of gender was observed ( $F(1, 78) = 0.760$ ;  $p = 0.386$ ; NS). Age was not found to be a significant covariate ( $F(1, 78) = 0.004$ ;  $p = 0.951$ ; NS).

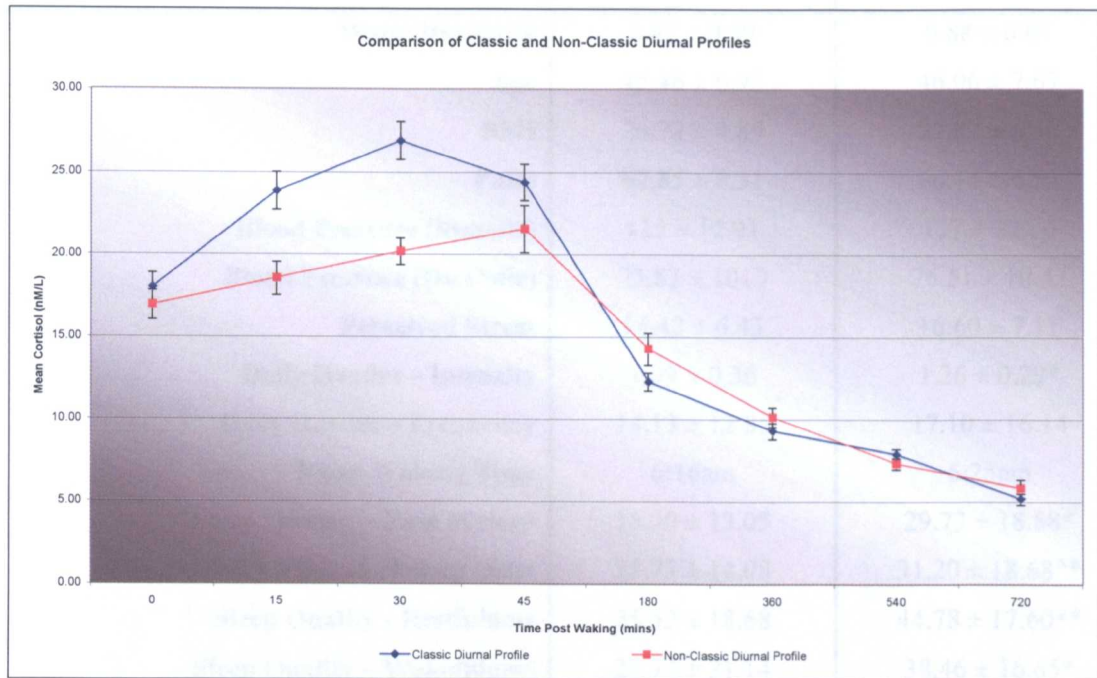
Taking these findings together, the results suggest that high and low waist-hip ratio groups differ in their expression of basal cortisol and that the cortisol level oscillated over the day. However, no differences in the shape of the curve were noted. The difference was based on the mean level of cortisol secreted over the diurnal period.

#### **5.4.4 Classifying Basal Profiles**

The composite classic and the non-classic profiles rated by 3 independent observers are shown in Figure 5.6 below. The analysis above was re-run based on these profile groups. This was conducted to determine differences between the profile groups and to highlight which individual characteristics are associated with deviations from the classic cortisol diurnal profile. A summary of the various characteristics is shown in Table 5.3.



#### 5.4.4.1 Basal Cortisol in, High /Low Waist-hip Ratio, Gender & Classic/Non-Classic Profile groups



**Figure 5.6:** Cortisol Diurnal Profiles by the Classic/Non-Classic Profile Groups (Means  $\pm$  SEM)

The difference between the profiles is most evident during the cortisol awakening response, with little difference observed for the subsequent diurnal activity. The differences between a ‘classic’ and ‘non-classic’ profile were confirmed when the indices of cortisol activity were reconsidered in each profile group. A significantly greater mean increase ( $F(1,74)=10.002$ ;  $p<0.01$ ), area under the curve with reference to zero (AUC) ( $F(1, 74)= 11.191$ ;  $p<0.01$ ), area under the curve with reference to the first sample (AURC) ( $F(1,74)= 11.565$ ;  $p<0.01$ ) and change from waking to 30minutes post waking (Change 0-30) ( $F(1,74)=15.455$ ;  $p<0.01$ ) was observed in the ‘classic’ profile compared with the ‘non-classic’ profile. The observation that a ‘non-classic’ profile was flattened was supported by greater mean cortisol across the 12hours of sampling and during the diurnal period post cortisol awakening response and greater concentration of cortisol 12hours post waking. However, these mean differences were not significant.

#### 5.4.4.2 Sleep Quality

Because the literature suggests that sleep quality can influence cortisol, this may explain the difference in the profile previously observed (Figure 5.6). The 12h post waking

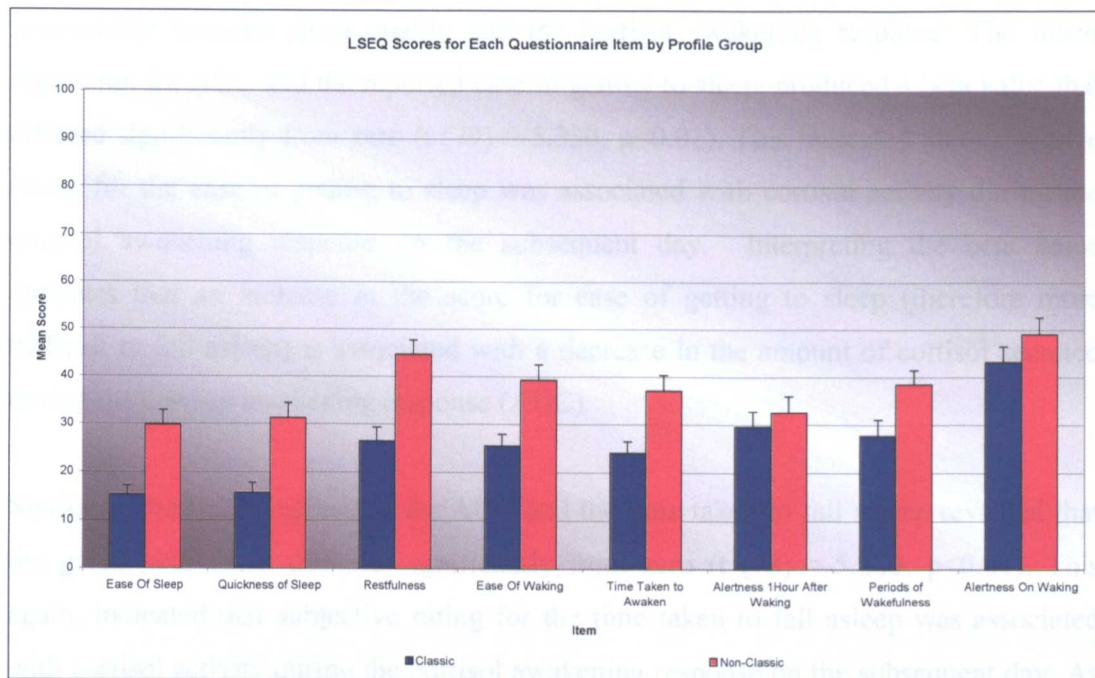
Variable	CLASSIC N = 48	NON-CLASSIC N = 35
Waist-Hip Ratio	0.87 ± 0.09	0.88 ± 0.07
Age	45.46 ± 6.93	46.06 ± 7.67
BMI	26.72 ± 4.89	27.87 ± 6.40
Pulse	62.85 ± 8.31	66.14 ± 6.53
Blood Pressure (Systolic)	115 ± 12.91	117 ± 22.70
Blood Pressure (Diastolic)	73.83 ± 10.10	76.51 ± 10.43
Perceived Stress	14.42 ± 6.43	16.60 ± 7.11
Daily Hassles – Intensity	1.09 ± 0.36	1.26 ± 0.29*
Daily Hassles – Frequency	14.13 ± 12.85	17.10 ± 16.14
Mean Waking Time	6:16am	6:25am
Sleep Quality – Ease of sleep	15.30 ± 13.05	29.73 ± 18.88*
Sleep Quality – Speed of sleep onset	15.73 ± 14.08	31.20 ± 18.68**
Sleep Quality - Restfulness	25.52 ± 18.68	44.78 ± 17.60**
Sleep Quality – Wakefulness	27.73 ± 21.14	38.46 ± 16.65*
Sleep Quality – Ease of Waking	25.52 ± 16.07	39.33 ± 19.18**
Sleep Quality – Alertness on waking	43.30 ± 21.95	49.11 ± 21.24
Sleep Quality – Alertness 1hr after waking	29.55 ± 21.11	32.60 ± 20.37
Sleep Quality – Time Taken to Awaken	24.04 ± 16.07	37.12 ± 18.45**
Plasma Glucose (mM/L)	5.09 ± 0.65	5.00 ± 0.62
Plasma insulin (mU/L)	5.97 ± 4.09	7.68 ± 6.02
Total Cholesterol (mM/L)	5.00 ± 0.94	5.27 ± 0.93
HDL (mM/L)	1.37 ± 0.31	1.34 ± 0.43
LDL (mM/L)	3.12 ± 0.83	3.23 ± 0.70
Triglycerides (mM/L)	1.15 ± 0.73	1.55 ± 0.93*
IL-6 (pg/ml)	1.44 ± 1.24	1.70 ± 1.17
CRP (mg/L)	2.41 ± 1.60	4.38 ± 4.82*
Adiponectin (ng/ml)	285.59 ± 187.12	272.88 ± 210.61
Insulin Resistance (HOMA)	1.42 ± 1.33	1.78 ± 1.70

**Table 5.3:** Summary of the Biological and Psychological Characteristics of a Classic & a Non-Classic Diurnal Profile (\*p<0.05 \*\*p<0.01) NOTE: a higher score for ratings on the LSEQ denotes poorer sleep

#### 5.4.4.2 Sleep Quality

Because the literature suggests that sleep quality can influence cortisol, this may explain the difference in the profile previously observed (Figure 5.6). The LSEQ measures

aspects of sleep quality with a high score indicating poor sleep quality. The scores for each item on the LSEQ were assessed collectively using a multivariate analysis of variance (MANOVA). Scores for each item by profile group are shown in Figure 5.7.



**Figure 5.7:** Scores on the LSEQ by the Classic/Non-Classic Profile Groups (Means  $\pm$  SEM)

There was a multivariate main effect of Profile group on LSEQ scores (Pillai's Trace  $F(8, 51) = 2.578$ ;  $p < 0.05$ ). Profile group had a significant main effect on all items on the LSEQ, except those measuring alertness (both upon waking and 1 hour post waking). Specifically, univariate analysis indicated there was a significant main effect of profile group on the ease of getting to sleep ( $F(1, 58) = 10.999$ ;  $p < 0.01$ ), the quickness of getting to sleep ( $F(1, 58) = 11.464$ ;  $p < 0.01$ ), restfulness of sleep ( $F(1, 58) = 8.545$ ;  $p < 0.01$ ), the number of periods of wakefulness reported ( $F(1, 58) = 3.890$ ;  $p < 0.05$ ), the ease of waking ( $F(1, 58) = 8.710$ ;  $p < 0.01$ ) and the time taken to wake ( $F(1, 58) = 5.957$ ;  $p < 0.05$ ). This pattern is clear in Figure 5.7 which shows that sleep quality was consistently reported to be poorer in individuals who exhibited a non-classic diurnal cortisol profile.

Further, cortisol, as indexed by the area under the curve with reference to zero (AUC), was a significant covariate (Pillai's Trace;  $F(8, 51) = 2.121$ ;  $p < 0.05$ ). Univariate analysis indicated that the ease of getting to sleep ( $F(1, 58) = 10.724$ ;  $p < 0.01$ ), quickness of sleep ( $F(1, 58) = 9.689$ ;  $p < 0.01$ ), reported restfulness of sleep ( $F(1, 58) = 7.212$ ;  $p < 0.01$ ) and reported number of periods of wakefulness ( $F(1, 58) = 8.402$ ;  $p < 0.01$ ) were

associated with cortisol during the cortisol awakening response (marked by the AUC index).

A linear regression was performed for each item with cortisol (AUC) to explore the relationship between sleep quality and the cortisol awakening response. The linear regression for AUC and the reported ease of getting to sleep, produced a beta value that differed significantly from zero ( $t(79) = -5.330$ ;  $p < 0.01$ ). This indicated that subjective rating for the ease of getting to sleep was associated with cortisol activity during the cortisol awakening response on the subsequent day. Interpreting the beta value indicates that an increase in the score for ease of getting to sleep (therefore more difficult to fall asleep) is associated with a decrease in the amount of cortisol secreted during the cortisol awakening response (AUC).

Similarly, the linear regression for AUC and the time taken to fall asleep revealed that the given beta value differed significantly from zero ( $t(78) = -5.280$ ;  $p < 0.01$ ). This again, indicated that subjective rating for the time taken to fall asleep was associated with cortisol activity during the cortisol awakening response on the subsequent day. As for ease of falling asleep, interpreting the beta value indicates that an increase in the score for time taken to get to sleep (therefore taking a longer period of time) is associated with a decrease in the amount of cortisol secreted during the cortisol awakening response (AUC).

For AUC and the reported restfulness of sleep, the given beta value was found to differ significantly from zero ( $t(78) = -4.458$ ;  $p < 0.05$ ). This indicated that subjective rating for restfulness of sleep was associated with cortisol activity during the cortisol awakening response on the subsequent day. Interpreting the beta value indicates that an increase in the score for restfulness sleep (therefore more restless sleep) is associated with a decrease in the amount of cortisol secreted during the cortisol awakening response (AUC).

For AUC and the number of periods of wakefulness, the given beta value was found to differ significantly from zero ( $t(78) = -4.881$ ;  $p < 0.05$ ). This indicated that subjective rating of the number of periods of wakefulness was associated with cortisol activity during the cortisol awakening response on the subsequent day. Interpreting the beta

value indicates that an increase in the score for the number of periods of wakefulness (therefore more periods of wakefulness) is associated with a decrease in the amount of cortisol secreted during the cortisol awakening response (AUC).

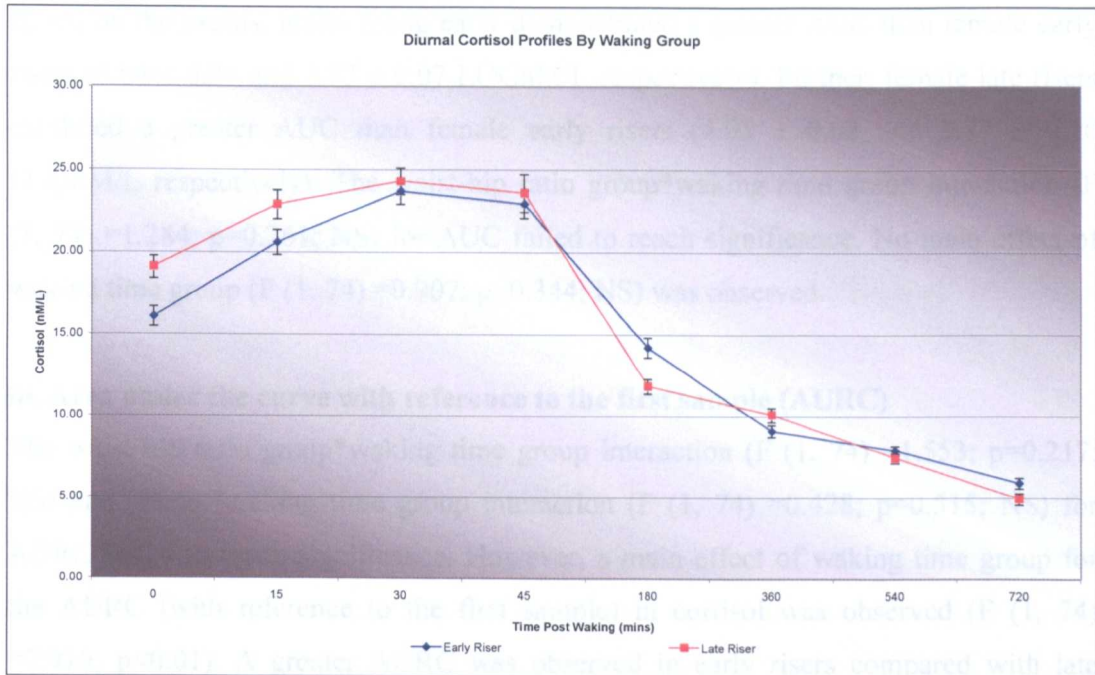
#### 5.4.4.3 Effect of Waking Time on the Basal Cortisol Profile

The waking times from each of the sampling three days were aggregated and assessed for consistency using a series of Pearson Bivariate correlations. Waking times demonstrated good consistency across the three days as illustrated in Table 5.2 below.

	Day One	Day Two	Day Three
Day One		0.765**	0.692**
Day Two	0.765**		0.783**
Day Three	0.692**	0.783**	

**Table 5.4:** Correlations for consistency across 3 sampling days for Waking Time (\*\* $p < 0.01$ )

To segregate the group into early and late risers, a median split performed. The median time of waking was calculated as being 6:23am. Therefore, individuals waking earlier than this time (or at this time) were classed as being early risers compared with individuals waking later than this time (late risers). Each index of cortisol activity was analysed individually. The composite diurnal profiles by waking time grouping are shown in Figure 5.8 below.



**Figure 5.8:** Basal Cortisol Diurnal Profile in early & later risers (Waking Group) (Means ± SEM)

A chi-square test for independence was performed to determine whether waking times differed between the classic and non-classic profiles. No association was observed between waking time group (early/late) and profile group (classic/non-classic) ( $\chi^2 = 0.009$ ;  $p=0.925$ ; NS).

The effect of waking time on the basal cortisol profile (without profile group as a between subjects factor) was explored using each index of cortisol activity.

#### **i. Mean Increase (MI)**

The waist-hip ratio group\*waking time group interaction ( $F(1, 74) = 1.659$ ;  $p=0.202$ ; NS) and gender\*waking time group interaction ( $F(1, 74) = 0.416$ ;  $p=0.521$ ; NS) for Mean Increase failed to reach significance. However, a main effect of waking time group for the mean increase in cortisol was observed ( $F(1, 74) = 7.749$ ;  $p<0.01$ ). A greater mean increase was observed in early risers compared with late risers ( $0.150 \pm 0.02$  and  $0.089 \pm 0.02$  LOGnM/L respectively).

#### **ii. Area under the curve with reference to zero (AUC)**

A significant gender\*waking group interaction was observed ( $F(1, 74) = 4.264$ ;  $p<0.05$ ). However, post hoc Bonferroni corrected t-tests failed to find any significant differences.

Based on the means, males rising early demonstrated a greater AUC than female early risers ( $4.06 \pm 0.06$  and  $3.77 \pm 0.07$  LOGnM/L respectively). Further, female late risers exhibited a greater AUC than female early risers ( $4.05 \pm 0.08$  and  $3.77 \pm 0.10$  LOGnM/L respectively). The waist-hip ratio group\*waking time group interaction ( $F(1, 74) = 1.284$ ;  $p = 0.261$ ; NS) for AUC failed to reach significance. No main effect of waking time group ( $F(1, 74) = 0.907$ ;  $p = 0.344$ ; NS) was observed.

### **iii. Area under the curve with reference to the first sample (AURC)**

The waist-hip ratio group\*waking time group interaction ( $F(1, 74) = 1.553$ ;  $p = 0.217$ ; NS) and gender\*waking time group interaction ( $F(1, 74) = 0.428$ ;  $p = 0.515$ ; NS) for AURC failed to reach significance. However, a main effect of waking time group for the AURC (with reference to the first sample) in cortisol was observed ( $F(1, 74) = 7.910$ ;  $p < 0.01$ ). A greater AURC was observed in early risers compared with late risers ( $0.315 \pm 0.31$  and  $0.189 \pm 0.32$  LOGnM/L respectively).

### **iv. Change 0-30**

The waist-hip ratio group\*waking time group interaction ( $F(1, 74) = 1.194$ ;  $p = 0.278$ ; NS) and gender\*waking time group interaction ( $F(1, 74) = 0.432$ ;  $p = 0.513$ ; NS) for the change in cortisol secretion between zero (immediately on waking) and 30minutes post waking (Change 0-30) failed to reach significance. However, a main effect of waking time group was observed ( $F(1, 74) = 7.808$ ;  $p < 0.01$ ). A greater change was observed in the early risers compared to the later risers ( $0.180 \pm 0.17$  and  $0.111 \pm 0.18$  LOGnM/L respectively).

### **v. Mean Diurnal Cortisol**

The waist-hip ratio group\*waking time group interaction ( $F(1, 74) = 4.22$ ;  $p = 0.518$ ; NS) and gender\*waking time group interaction ( $F(1, 74) = 0.654$ ;  $p = 0.421$ ; NS) for the diurnal mean of cortisol failed to reach significance. However, a main effect of waking time group for mean diurnal cortisol secretion was observed ( $F(1, 74) = 4.945$ ;  $p < 0.05$ ). A greater diurnal mean cortisol was observed in early risers compared with late risers ( $0.926 \pm 0.17$  and  $0.873 \pm 0.17$  LOGnM/L respectively).

No main effects for the Day Mean of cortisol were observed.

**vi. Day Difference 0-12hours Post Waking**

A significant gender\*waking time group interaction was observed ( $F(1, 74) = 4.423$ ;  $p < 0.05$ ). Post hoc analyses revealed that a greater change in cortisol secretion between waking and the final sample was observed in female later risers compared with female early risers ( $p = 0.001$ ) ( $0.704 \pm 0.04$  and  $0.427 \pm 0.06$  LOGnM/L respectively). The waist-hip ratio group\*waking time group interaction for the difference in cortisol between waking and 12hours post waking, failed to reach significance ( $F(1, 74) = 0.081$ ;  $p = 0.776$ ; NS).

A main effect of waking time group for mean diurnal cortisol secretion was observed ( $F(1, 74) = 7.566$ ;  $p < 0.01$ ). A greater difference in cortisol was observed in late risers compared to early risers ( $0.616 \pm 0.41$  and  $0.456 \pm 0.41$  LOGnM/L respectively)

**vii. Final Sample**

A trend for a significant gender \*waking group interaction was observed ( $F(1, 74) = 3.022$ ;  $p = 0.086$ ). Post hoc analyses revealed that a reduced cortisol concentration at the final sample was observed in female later risers compared with male later risers ( $p = 0.008$ ) and compared with female early risers ( $p = 0.012$ ) (female late:  $0.561 \pm 0.03$  LOGnM/L versus male late:  $0.721 \pm 0.04$  LOGnM/L and female early:  $0.716 \pm 0.05$  LOGnM/L respectively). The waist-hip ratio group\*waking time group interaction ( $F(1, 74) = 0.363$ ;  $p = 0.549$ ; NS) failed to reach significance.

A trend for a main effect of waking time group for the final sample (Sample H) in cortisol was observed ( $F(1, 74) = 3.548$ ;  $p = 0.064$ ). A greater cortisol concentration was observed in early risers compared to the later risers ( $0.724 \pm 0.31$  and  $0.640 \pm 0.32$  LOGnM/L respectively).

**5.4.4.4 Daily Hassles & Perceived Stress**

Because the literature suggests that, like sleep quality, daily hassles and perceived stress can influence cortisol, this may explain the difference in the profile previously observed (Figure 5.6). Scores for daily hassles from the Daily Hassles Scale and perceived stress from the Perceived Stress Scale, were analysed using a 2x2x2 ANOVA with waist-hip ratio, gender and profile group as between subjects factors.



**(i) Influence of Daily Hassles on the Basal Cortisol Profile**

Two scores are obtained from the daily hassles scale i. the intensity of the hassle experienced and ii. frequency of the hassles encountered.

**- Intensity**

Day Mean of cortisol was a significant covariate in the analysis of the subjective intensity of the daily hassles encountered ( $F(1, 73) = 5.359; p < 0.05$ ). Using a linear regression, the given beta value for the relationship between the subjective mean intensity of daily hassles and mean cortisol was found to differ significantly from zero ( $t(82) = -2.303; p < 0.05$ ). This indicated that subjective rating of the intensity of daily hassles experienced was associated with mean cortisol activity over the diurnal period. Interpreting the beta value indicates that an increase in the subjective intensity of daily hassles is associated with a decrease in the mean of cortisol secreted (Day Mean).

The waist-hip ratio group\*profile group interaction ( $F(1, 73) = 0.859; p = 0.357; NS$ ) and gender\*profile group interaction ( $F(1, 73) = 1.343; p = 0.250; NS$ ) for subjective intensity of daily hassles failed to reach significance. However, a main effect of profile group was observed ( $F(1, 73) = 6.200; p < 0.05$ ). A greater mean intensity of hassles was noted in the non-classic profile group compared with the classic profile group ( $1.278 \pm 0.06$  and  $1.093 \pm 0.05$  respectively).

**- Frequency**

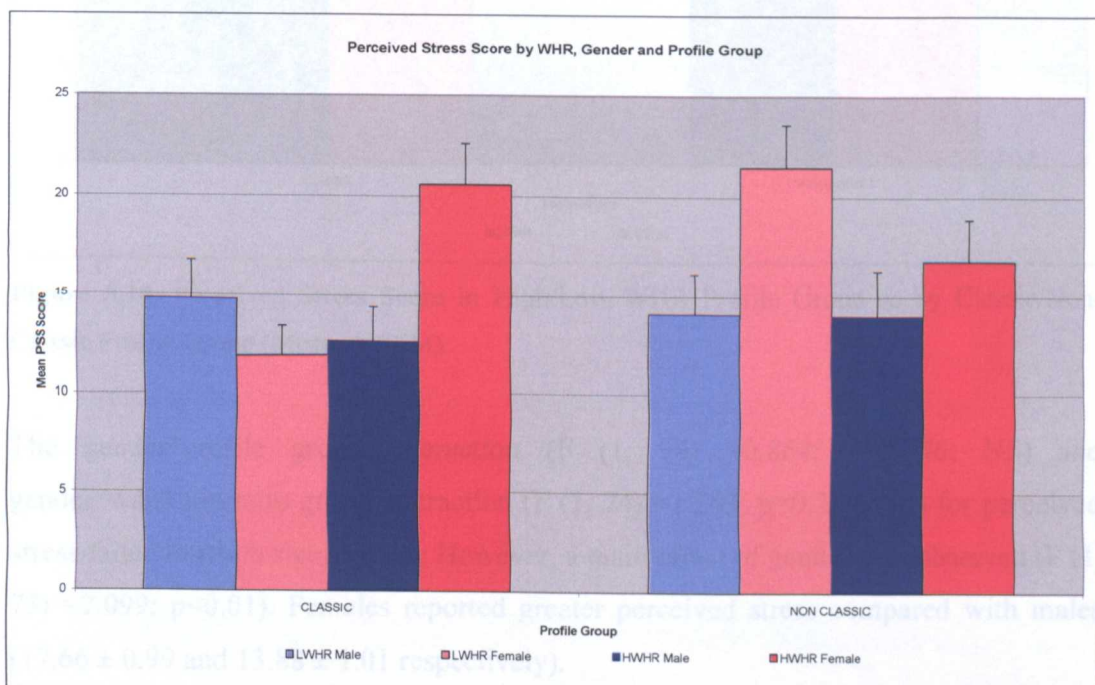
No main effects or interactions for the frequency of daily hassles were observed.

**(b) Perceived Stress**

Scores from the Perceived Stress Scale were analysed using a  $2 \times 2 \times 2$  ANOVA with waist-hip ratio, gender and profile group as between subjects factors.

Age was a significant covariate in the analysis of perceived stress score ( $F(1, 74) = 4.405; p < 0.05$ ). However, using a linear regression, the given beta value for the relationship between the subjective perceived stress and age did not significantly differ from zero ( $t(82) = -1.186; p = 0.239; NS$ ). This suggests that the relationship between age and perceived stress score is non-significant.

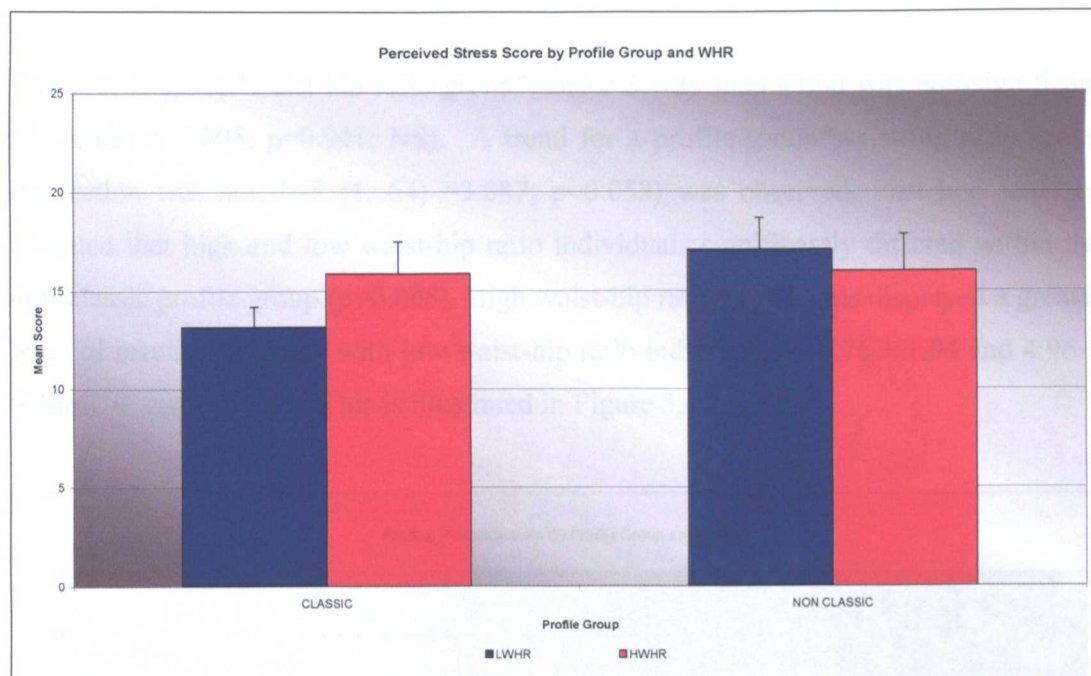
A significant waist-hip ratio\*gender\*profile group interaction was observed ( $F(1, 73) = 7.099$ ;  $p < 0.05$ ). Post hoc analyses revealed a significant difference between classic and non-classic profile groups in low waist-hip ratio females ( $p = 0.005$ ). Low waist-hip ratio females displaying a non-classic profile exhibited a significantly greater perceived stress score compared with low waist-hip ratio females displaying a classic profile ( $20.22 \pm 2.51$  and  $12.00 \pm 1.41$  respectively). In addition, high waist-hip ratio females displaying a classic profile also significantly differed from low waist-hip ratio females in the same group ( $p = 0.002$ ). High waist-hip ratio females again exhibited significantly greater perceived stress scores compared with low waist-hip ratio females ( $20.56 \pm 2.22$  and  $12.00 \pm 1.41$  respectively). This is illustrated in Figure 5.9 below.



**Figure 5.9:** Perceived Stress Score in High/Low WHR males/females & by Classic/Non-Classic Profile Group (Means  $\pm$  SEM)

A significant waist-hip ratio group\*profile group interaction was observed ( $F(1, 74) = 4.077$ ;  $p < 0.05$ ). Post hoc analysis revealed that low waist-hip ratio individuals displaying a classic diurnal profile, differed from low waist-hip ratio individuals displaying a non-classic diurnal profile. However, this was non-significant ( $p = 0.045$  Bonferroni corrected). Low waist-hip ratio individuals displaying a non-classic diurnal profile reported greater perceived stress than low waist-hip ratio individuals displaying

a classic diurnal profile ( $17.11 \pm 1.59$  and  $13.19 \pm 0.99$  respectively). This is illustrated in Figure 5.10 below.



**Figure 5.10:** Perceived Stress Score in High/Low WHR Profile Group & by Classic/Non-Classic Profile Group (Means  $\pm$  SEM)

The gender\*profile group interaction ( $F(1, 74) = 0.864$ ;  $p = 0.356$ ; NS) and gender\*waist-hip ratio group interaction ( $F(1, 74) = 1.243$ ;  $p = 0.269$ ; NS) for perceived stress failed to reach significance. However, a main effect of gender was observed ( $F(1, 73) = 7.099$ ;  $p < 0.01$ ). Females reported greater perceived stress compared with males ( $17.66 \pm 0.99$  and  $13.88 \pm 1.01$  respectively).

#### 5.4.4.5 Blood Biomarkers & DHEA

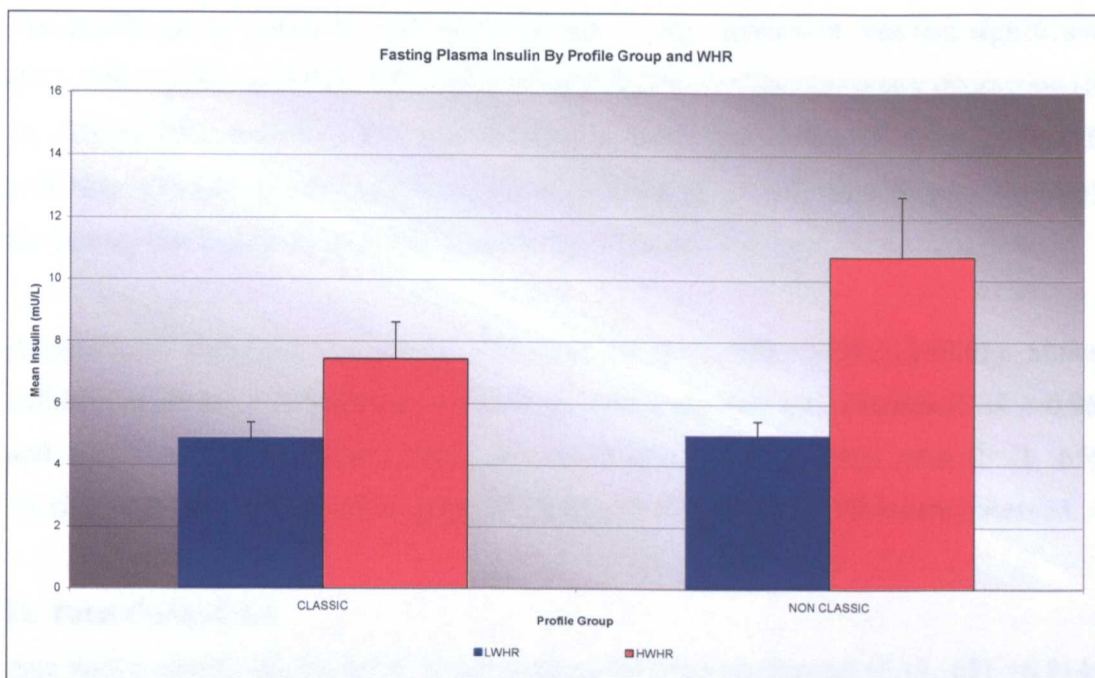
As previous literature suggests that metabolic syndrome is a neuroendocrine disorder, the measured concentration of each biomarker may explain the difference between the classic and non-classic diurnal profiles. Each biomarker was analysed using a 2x2x2 ANOVA with waist-hip ratio, gender and profile group as a between subjects factors. Due to missing data from unsuccessful venepuncture and removal of extreme outliers, between 74 and 76 observations out of 83 were included in the analysis.

A significant main effect of gender was observed ( $F(1, 69) = 14.813$ ;  $p < 0.01$ ). Males exhibited a greater concentration of insulin compared with females ( $5.34 \pm 0.96$  and  $4.31 \pm 0.95$  mU/L respectively). Further, a main effect of waist-hip ratio was observed

### i. Fasting Plasma Insulin

Age ( $F(1, 64) = 0.043$ ;  $p=0.836$ ; NS) and cortisol (Day Mean) ( $F(1, 64) = 0.028$ ;  $p=0.867$ ; NS) as covariates were non-significant.

The profile group\*waist-hip ratio group\*gender 3 way interaction was non-significant ( $F(1, 65) = 0.005$ ;  $p=0.941$ ; NS). A trend for a profile group\*waist-hip ratio group interaction was noted ( $F(1, 64) = 3.887$ ;  $p=0.053$ ) was observed. Post hoc analyses revealed that high and low waist-hip ratio individuals significantly differed within the non-classic profile group ( $p=0.005$ ). High waist-hip ratio individuals displayed a greater level of insulin compared with low waist-hip ratio individuals ( $10.76 \pm 1.94$  and  $4.96 \pm 0.48$  mU/L respectively). This is illustrated in Figure 5.11 below.



**Figure 5.11:** Fasting Plasma Insulin Score in High/Low WHR Profile Group & by Classic/Non-Classic Profile Group (Means  $\pm$  SEM)

The gender\*profile group interaction ( $F(1, 65) = 2.036$   $p=0.158$ ; NS) and waist-hip ratio\*gender interaction ( $F(1, 65) = 1.347$ ;  $p=0.250$ ; NS) for fasting plasma insulin all failed to reach significance.

A significant main effect of gender was observed ( $F(1, 69) = 14.813$ ;  $p<0.01$ ). Males exhibited a greater concentration of insulin compared with females ( $5.34 \pm 0.96$  and  $4.81 \pm 0.95$  mU/L respectively). Further, a main effect of waist-hip ratio was observed

( $F(1, 64) = 12.831$ ;  $p < 0.01$ ). Higher insulin was observed in high waist-hip ratio individuals compared with low waist-hip ratio individuals ( $9.08 \pm 0.82$  and  $5.05 \pm 0.73$  mU/L respectively). No main effect of profile group ( $F(1, 65) = 0.508$ ;  $p = 0.478$ ; NS) was observed.

### ii. Fasting Plasma Glucose

Age significantly co-varied with fasting plasma glucose ( $p = 0.037$ ). However, using a linear regression, the given beta value for the relationship between the blood glucose and age did not differ significantly from zero ( $t(76) = 1.631$ ;  $p = 0.107$ ; NS). The relationship between age and blood glucose was non-significant. Cortisol (Day Mean) was not a significant covariate ( $F(1, 65) = 0.000$ ;  $p = 0.994$ ; NS)

The profile group\*waist-hip ratio group\*gender 3 way interaction was non-significant ( $F(1, 65) = 0.005$ ;  $p = 0.941$ ; NS). The profile group\*waist-hip ratio group interaction ( $F(1, 65) = 0.001$ ;  $p = 0.981$ ; NS), gender\*profile group interaction ( $F(1, 65) = 0.125$ ;  $p = 0.724$ ; NS) and waist-hip ratio\*gender interaction ( $F(1, 65) = 0.458$ ;  $p = 0.501$ ; NS) for fasting blood glucose all failed to reach significance.

A significant main effect of gender was observed ( $F(1, 69) = 14.813$ ;  $p < 0.01$ ). Males exhibited a greater concentration of blood glucose compared with females ( $5.34 \pm 0.96$  and  $4.81 \pm 0.95$  mM/L respectively). No main effects of waist-hip ratio ( $F(1, 65) = 1.139$ ;  $p = 0.290$ ; NS) or profile group ( $F(1, 65) = 0.508$ ;  $p = 0.478$ ; NS) were observed.

### iii. Total Cholesterol

Age was a significant covariate in the analysis of total cholesterol ( $F(1, 68) = 6.814$ ;  $p < 0.05$ ). Using a linear regression, the given beta value for the relationship between total cholesterol and age was found to differ significantly from zero ( $t(76) = 2.729$ ;  $p < 0.01$ ). This indicated that age was associated with changes in cholesterol. Interpreting the beta value indicates that an increase in age is associated with an increase in total cholesterol.

The profile group\*waist-hip ratio group\*gender 3 way interaction was non-significant ( $F(1, 64) = 0.006$ ;  $p = 0.937$ ; NS). The profile group\*waist-hip ratio group interaction ( $F(1, 64) = 1.268$ ;  $p = 0.264$ ; NS), gender\*profile group interaction ( $F(1, 64) = 0.789$ ;

$p=0.378$ ; NS) and waist-hip ratio\*gender interaction ( $F(1, 64) = 1.230$ ;  $p=0.272$ ; NS) for total cholesterol all failed to reach significance.

A trend for a main effect of profile group was observed ( $F(1, 64) = 3.215$ ;  $p=0.078$ ). Higher cholesterol was exhibited by individuals displaying a non-classic cortisol profile compared with a classic profile ( $5.37 \pm 0.18$  and  $4.91 \pm 0.16$  mM/L respectively). No main effect of waist-hip ratio group ( $F(1, 64) = 0.035$ ;  $p=0.852$ ; NS) or gender ( $F(1, 64) = 0.063$ ;  $p=0.802$ ; NS) was observed.

#### **iv. Low Density Lipoproteins (LDL)**

Age ( $F(1, 64) = 1.109$ ;  $p=0.296$ ; NS) and cortisol (Day Mean) ( $F(1, 64) = 1.143$ ;  $p=0.239$ ; NS) were non-significant covariates.

The profile group\*waist-hip ratio group\*gender 3 way interaction was non-significant ( $F(1, 64) = 0.397$ ;  $p=0.531$ ; NS). The profile group\*waist-hip ratio group interaction ( $F(1, 64) = 0.833$ ;  $p=0.365$ ; NS), gender\*profile group interaction ( $F(1, 64) = 0.464$ ;  $p=0.498$ ; NS) and waist-hip ratio\*gender interaction ( $F(1, 64) = 0.781$ ;  $p=0.380$ ; NS) for LDL all failed to reach significance. No main effect of waist-hip ratio ( $F(1, 64) = 0.014$ ;  $p=0.905$ ; NS), gender ( $F(1, 64) = 0.003$ ;  $p=0.957$ ; NS) or profile group ( $F(1, 64) = 2.224$ ;  $p=0.141$ ; NS) was observed.

#### **v. High Density Lipoproteins (HDL)**

Age was found to significantly co-vary with HDL concentration ( $F(1, 68) = 9.354$ ;  $p<0.01$ ). Using a linear regression, the given beta value for the relationship between HDL and age was found to differ significantly from zero ( $t(76) = 3.036$ ;  $p<0.01$ ). This indicated that age was associated with changes in HDL. Interpreting the beta value indicates that an increase in age is associated with an increase in HDL.

The profile group\*waist-hip ratio group\*gender 3 way interaction was non-significant ( $F(1, 64) = 0.450$ ;  $p=0.505$ ; NS). The profile group\*waist-hip ratio group interaction ( $F(1, 64) = 1.093$ ;  $p=0.300$ ; NS), gender\*profile group interaction ( $F(1, 64) = 1.891$ ;  $p=0.174$ ; NS) and waist-hip ratio\*gender interaction ( $F(1, 64) = 0.287$ ;  $p=0.594$ ; NS) for HDL all failed to reach significance

A main effect of gender on HDL was observed ( $F(1, 68) = 12.617; p < 0.01$ ). Females exhibited a significantly greater HDL concentration compared with males ( $1.478 \pm 0.05$  and  $1.213 \pm 0.05$  mM/L respectively). A trend for a main effect of waist-hip ratio on was also observed ( $F(1, 64) = 3.261; p = 0.076$ ). No main effect of profile group was observed ( $F(1, 64) = 1.040; p = 0.312; NS$ ).

#### vi. Triglycerides

Age ( $F(1, 64) = 1.672; p = 0.201; NS$ ) and cortisol (Day Mean) ( $F(1, 64) = 0.414; p = 0.512; NS$ ) were not identified as significant covariates.

A trend was observed for a profile group\*waist-hip ratio\*gender three way interaction ( $F(1, 64) = 3.095; p = 0.083$ ). Post hoc analyses revealed a significant difference the measures concentration of triglycerides between high waist-hip ratio males demonstrating a classic profile and low waist-hip ratio males in the same profile group ( $p = 0.003$ ) High waist-hip ratio males exhibited a greater level of triglycerides compare with low waist-hip ratio males ( $1.87 \pm 0.30$  and  $0.79 \pm 0.08$  mM/L respectively). The profile group\*waist-hip ratio group interaction ( $F(1, 64) = 0.181; p = 0.672; NS$ ), gender\*profile group interaction ( $F(1, 64) = 0.991; p = 0.323; NS$ ) and waist-hip ratio\*gender interaction ( $F(1, 64) = 1.457; p = 0.232; NS$ ) for triglycerides all failed to reach significance.

A main effect of waist-hip ratio group was observed ( $F(1, 68) = 5.996; p < 0.05$ ). High waist-hip ratio individuals exhibited a greater concentration of triglycerides than low waist-hip ratio individuals ( $1.56 \pm 0.13$  and  $1.16 \pm 0.12$  mM/L respectively). A main effect of profile group was also observed ( $F(1, 64) = 4.839; p < 0.05$ ). A greater concentration of triglycerides was noted in those displaying a non-classic cortisol diurnal profile compared with those exhibiting a classic profile ( $1.591 \pm 0.15$  and  $1.131 \pm 0.13$  mM/L respectively). Finally, a main effect of gender was observed ( $F(1, 64) = 12.074; p < 0.01$ ). A greater concentration of triglycerides was noted in males compared with females ( $1.674 \pm 0.13$  and  $1.048 \pm 0.12$  mM/L respectively).

#### vii. Interleukin-6

Cortisol (AUC) was a significant covariate in the analysis of IL-6 ( $F(1, 63) = 4.718; p < 0.05$ ). Using a linear regression, the given beta value for the relationship between IL-

6 and cortisol (AUC) was found to differ significantly from zero ( $t(69) = -0.253$ ;  $p < 0.05$ ). This indicated that cortisol is associated with changes in IL-6. Interpreting the beta value indicates that an increase in IL-6 is associated with a decrease in cortisol (AUC) secreted during the awakening response.

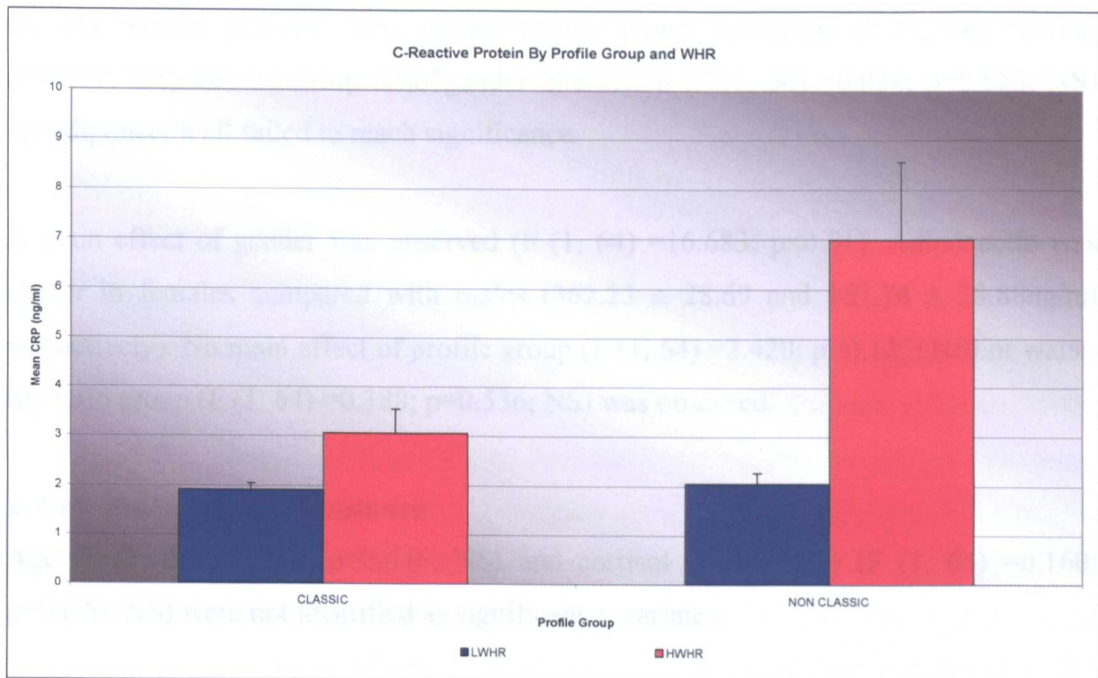
The profile group\*waist-hip ratio group\*gender 3 way interaction was non-significant ( $F(1, 61) = 0.842$ ;  $p = 0.363$ ; NS). The profile group\*waist-hip ratio group interaction ( $F(1, 61) = 0.180$ ;  $p = 0.673$ ; NS), gender\*profile group interaction ( $F(1, 61) = 0.027$ ;  $p = 0.871$ ; NS) and waist-hip ratio\*gender interaction ( $F(1, 61) = 0.656$ ;  $p = 0.421$ ; NS) for IL-6 all failed to reach significance. A main effect of waist-hip ratio was observed ( $F(1, 66) = 11.912$ ;  $p < 0.01$ ). High waist-hip ratio individuals exhibited a greater concentration of IL-6 than low waist-hip ratio individuals ( $2.02 \pm 0.21$  and  $1.09 \pm 0.19$  pg/ml respectively). No main effect of gender ( $F(1, 61) = 1.580$ ;  $p = 0.214$ ; NS) or profile group ( $F(1, 61) = 0.045$ ;  $p = 0.832$ ; NS) was observed.

#### **viii. C-Reactive Protein**

Cortisol (AUC) was a significant covariate in the analysis of CRP ( $F(1, 69) = 13.115$ ;  $p < 0.05$ ). Using a linear regression, the given beta value for the relationship between CRP and AUC was found to differ significantly from zero ( $t(75) = -3.184$ ;  $p < 0.01$ ). This indicated that cortisol is associated with changes in CRP. Interpreting the beta value indicates that an increase in CRP was associated with a decrease in cortisol (AUC) secreted during the awakening response.

The profile group\*waist-hip ratio group\*gender 3 way interaction was non-significant ( $F(1, 64) = 0.05$ ;  $p = 0.816$ ; NS). A significant profile group\*waist-hip ratio group interaction was observed ( $F(1, 64) = 8.243$ ;  $p < 0.01$ ). Post hoc analysis revealed a significant difference between high and low waist-hip ratio individuals within the non-classic profile group ( $p = 0.002$ ). High waist-hip ratio individuals demonstrated greater CRP than low waist-hip ratio individuals ( $6.84 \pm 1.01$  and  $2.20 \pm 0.94$  mg/L respectively) (Figure 5.12).





**Figure 5.12:** C-Reactive Protein Score in High/Low WHR Profile Group & by Classic/Non-Classic Profile Group (Means  $\pm$  SEM)

The gender\*profile group interaction ( $F(1, 64) = 0.046$ ;  $p = 0.830$ ; NS) and waist-hip ratio\*gender interaction ( $F(1, 64) = 0.884$ ;  $p = 0.351$ ; NS) for CRP all failed to reach significance.

A main effect of waist-hip ratio group was observed ( $F(1, 69) = 13.115$ ;  $p < 0.01$ ). High waist-hip ratio individuals exhibited a greater mean concentration of CRP than low waist-hip ratio individuals ( $4.86 \pm 0.53$  and  $2.07 \pm 0.47$  mg/L respectively). Further, a significant main effect of profile group was observed ( $F(1, 64) = 4.109$ ;  $p < 0.05$ ). Those exhibiting a non-classic cortisol diurnal profile also exhibited elevated C-reactive protein compared with those exhibiting a classic cortisol diurnal profile ( $4.31 \pm 0.58$  and  $2.62 \pm 0.50$  mg/L respectively). No main effect of gender was observed ( $F(1, 64) = 0.935$ ;  $p = 0.337$ ; NS).

#### ix. Adiponectin

Age ( $F(1, 64) = 1.855$ ;  $p = 0.178$ ; NS) and cortisol (Day Mean) ( $F(1, 64) = 0.338$ ;  $p = 0.563$ ; NS) were not identified as significant covariates.

The profile group\*waist-hip ratio group\*gender 3 way interaction was non-significant ( $F(1, 64) = 0.021$ ;  $p = 0.885$ ; NS). The profile group\*waist-hip ratio group interaction ( $F(1, 64) = 0.021$ ;  $p = 0.885$ ; NS).

(1, 64) =0.000; p=0.987; NS), gender\*profile group interaction (F (1, 64) =0.196; p=0.659; NS) and waist-hip ratio\*gender interaction (F (1, 64) =0.034; p=0.853; NS) for adiponectin all failed to reach significance.

A main effect of gender was observed (F (1, 64) =16.683; p<0.01). Adiponectin was higher in females compared with males (362.23 ± 28.69 and 187.74 ± 28.88ng/ml respectively). No main effect of profile group (F (1, 64) =2.420; p=0.125; NS) or waist-hip ratio group (F (1, 64) =0.388; p=0.536; NS) was observed.

#### **x. Calculated Insulin Resistance**

Age (F (1, 64) =0.213; p=0.646; NS) and cortisol (Day Mean) (F (1, 64) =0.160; p=0.690; NS) were not identified as significant covariates.

A main effect of gender was observed (F (1, 64) =7.028; p<0.05). A greater vulnerability to insulin resistance was demonstrated in males compared with females (2.12 ± 0.24 and 1.22 ± 0.24 respectively). Further, a main effect of waist-hip ratio group was observed (F (1, 64) =9.914; p<0.01). A greater level of insulin resistance was observed in high waist-hip ratio individuals compared with low waist-hip ratio individuals (2.20 ± 0.25 and 1.14 ± 0.23 respectively). No main effect of profile group was observed (F (1, 64) =1.260; p=0.266; NS).

#### **xi. Dehydroepiandrosterone (DHEA)**

Age (F (1, 64) =0.273; p=0.603; NS) and cortisol (Day Mean) (F (1, 64) =0.007; p=0.933; NS) were not identified as significant covariates.

The profile group\*waist-hip ratio group\*gender 3 way interaction was non-significant (F (1, 62) = 0.060; p=0.807; NS). The profile group\*waist-hip ratio group interaction (F (1, 62) =0.036; p=0.849; NS), gender\*profile group interaction (F (1, 62) =0.020; p=0.888; NS) and waist-hip ratio\*gender interaction (F (1, 62) =0.000; p=0.983; NS) for dehydroepiandrosterone failed to reach significance.

A main effect of waist-hip ratio group was observed (F (1, 62) =6.142; p<0.05). A greater concentration of DHEA was observed in high waist-hip ratio individuals than in low waist-hip ratio individuals (2.759 ± 0.47 and 1.147 ± 0.41nM/L respectively). No

main effect of profile group ( $F(1, 62) = 0.284$ ;  $p = 0.596$ ; NS) or gender ( $F(1, 62) = 1.661$ ;  $p = 0.202$ ; NS) was observed.

#### 5.4.5 Summary of Results

- **Basal Cortisol Diurnal Profile by Waist-hip Ratio & Gender**

Basal cortisol profiles did not differ significantly in relation to waist-hip ratio or gender, yet despite similar profiles, high waist-hip ratio individuals exhibited lower mean cortisol over the diurnal profile than low waist-hip ratio individual's evidence by a trend for a main effect of waist-hip ratio group. Age was not found to be related to cortisol secretion and no gender differences were observed in the cortisol profiles.

- **Waist-Hip ratio & Metabolic Factors**

Greater insulin and calculated degree of insulin resistance, triglycerides, C - reactive protein, IL-6 and DHEA were observed in high waist-hip ratio individuals compared with low waist-hip ratio individuals.

- **Classic & Non-Classic Diurnal Profiles**

The non-classic profile differed significantly to the classic profile evidenced by differences in a number of cortisol indices. A greater mean increase, AURC, AUC and change 0-30 were observed in the classic profile compared with the non-classic profile. However, mean cortisol was greater in the non-classic profile group who also showed a greater cortisol concentration at the final sample. The high incidence of non-classic profiles was distributed in similar proportions across the waist-hip ratio and gender groups. The non-classic profiles were associated with poor sleep quality (LSEQ), subjective intensity of daily hassles experienced (Daily Hassles) and elevated CRP and triglycerides. A trend for greater cholesterol concurrent with a non-classic profile was also observed.

### 5.5 Discussion

#### 5.5.1 General Aims

This study aimed to explore basal salivary cortisol activity in individuals with central obesity (high waist-hip ratio) compared to peripherally obese (low waist-hip ratio) or

lean individuals in the age range 35-65 years. Profiles were initially compared for cortisol activity with respect to waist-hip ratio and gender. Assessment of the prevalence of a classic diurnal profile as opposed to a non-classic profile and possible factors associated with the profile were explored. Metabolic syndrome symptomology (biomarkers associated with central obesity), sleep quality, waking time and perceived stress (both daily hassles and perceived stress score were explored in terms of waist-hip ratio, gender and cortisol profile group (classic/non-classic).

## **5.5.2 Basal Salivary Cortisol in High/Low WHR Males/Females**

### **5.5.2.1 Differences in Basal Cortisol**

Analysis of the basal salivary cortisol data in relation to central obesity did not demonstrate any differences in the shape of the diurnal profile between high waist-hip ratio (centrally obese) individuals and low waist-hip ratio (lean and peripherally obese) individuals. However, individuals with central obesity did demonstrate lower mean cortisol across the diurnal profile.

The literature reviewed in Chapter One suggested that salivary cortisol levels are lower in the centrally obese. This is supported by the findings of the current study. This can be explained by the proposal that underlying HPA dysregulation permits over-secretion of cortisol. However, over secretion is not detectable in plasma or saliva during the diurnal period because cortisol clearance capability is enhanced (Lottenberg et al., 1998; Strain et al., 1980; Ljung et al., 1996; Marin et al., 1992). The proposed cortisol hypersecretion is primarily evidenced by elevated urinary cortisol excretory metabolites (Lottenberg et al., 1998). Because of this, a large number of studies have reported no difference or often lower basal cortisol secretion during the diurnal period in the centrally obese compared to lean or peripherally obese individuals (high versus low waist-hip ratio). The current study found that mean salivary cortisol was reduced in those with central obesity compared with low waist-hip ratio individuals in support of previous findings. However, as urinary cortisol was not sampled to examine the excretion of cortisol metabolites, it cannot be ascertained if an enhanced clearance capability was exhibited by individuals with central obesity in the study presented in this chapter.

An alternative explanation for the observed differences in basal cortisol secretion derives from the idea that there exists a nocturnal circadian trough in cortisol activity

(Andrew et al., 1998; Ljung et al., 2000; Phillips et al., 2000). An increase in cortisol during this period of nocturnal low-level activity produces a compensatory reduction in the peak level of cortisol (i.e. the cortisol awakening response). This method of response means that there is no detected change in the overall level of cortisol activity or an observed lower than normal level of activity which is again due to a disrupted HPA axis. The findings of the current study offer some support to for this proposal but it is not possible to verify the existence of a circadian trough, responsible for this compensatory response, as nocturnal cortisol was not assessed. Further, mean cortisol secreted during the cortisol response to waking in the current study was lower in high waist-hip ratio individuals (albeit not significantly). This would concur with a proposed reduction in the peak level of cortisol during the circadian profile (Van Cauter et al., 1996). Therefore, those exhibiting central obesity will exhibit a blunted cortisol awakening response (Andrew et al., 1998; Ljung et al., 2000; Phillips et al., 2000) due to elevated nocturnal cortisol. The findings of the current study partially support a disrupted HPA regulation yet it is not possible to verify the existence of a circadian trough nor can it be verified by an increase in the excretion of urinary cortisol metabolites as these were not measured. Therefore, the claim that those with central obesity exhibit lower cortisol due to HPA dysregulation in conjunction with an enhanced clearance rate requires further evidence beyond the scope of this thesis. Based on the current findings, basal cortisol secretion in those with central obesity is reduced compared with low waist-hip ratio individuals. To determine what this reduction is attributed to requires further research with measures of cortisol clearance.

#### **5.5.2.2 Biomarkers of Obesity & the Metabolic Syndrome**

The possibility that the metabolic syndrome is a neuroendocrine disorder is disputed (Bjorntorp & Rosmond, 2000; Rosmond et al, 1998; Bahr et al., 2002). The current study aimed to explore associations between cortisol activity, central obesity and the various biomarkers that are implicated in the symptomology of the metabolic syndrome. Hence, a number of biomarkers were also assessed in addition to the assessment of salivary cortisol in relation to central obesity. These included insulin, glucose (including the calculated degree of insulin resistance), cholesterol, low-density lipoprotein, high-density lipoprotein, triglycerides, interleukin-6, C - reactive protein and adiponectin. In the current study, measured insulin (and calculated degree of insulin resistance), IL-6, C-RP, triglycerides, HDL differed between waist-hip ratio groups. For all biomarkers

(except HDL which was reduced) the measured level was elevated in high waist-hip ratio individuals compared with low waist-hip ratio individuals. This is in accordance with previous research findings and supports the postulation that the centrally obese are of a poorer health status when compared to those exhibiting peripheral obesity (Bjorntorp and Rosmond, 2000). Indeed individuals with peripheral obesity did not demonstrate any of the associated health complications often associated with central obesity. This supports of the view that the type and distribution of body fat is important for the prognosis of long-term health consequences. Those exhibiting central obesity are at increased risk of insulin resistance and diabetes, cardiovascular disease and stroke (Hartz et al., 1984).

In terms of the role of cortisol in the expression of central obesity, minimal supporting evidence was presented in the current study. Indices of cortisol were included as covariates (AUC and Day Mean) to assess the potential for cortisol to interact with other metabolic syndrome symptoms in the expression of the metabolic syndrome. Cortisol only found to significantly correlate with C - reactive protein and IL-6 (inflammatory immune markers), with no observed co-variance with any of the remaining biomarkers. The suggestion that the Metabolic Syndrome may indeed be a neuroendocrine disorder (Bjorntorp and Rosmond, 2000; Gale et al., 2002; Rosmond and Bjorntorp, 2001; Khani and Tayek, 2001) remains largely unsupported in the current study. However, it may be that the relationship between cortisol and the metabolic syndrome is far more complex than this assessment allowed. Gradual changes in cortisol and metabolic parameters as a result of chronic stress and elevated basal cortisol over a much longer period may be characteristic of the neuroendocrine hypothesis (Bjorntorp and Rosmond, 2001). Confirmation of this would require a more longitudinal assessment in a study much larger than this thesis permits.

DHEA was found to be elevated in high waist-hip ratio individuals. Previous research has suggested that an inverse relationship exists between DHEA and BMI (De Pergola et al., 1996) but is largely conflicting with some studies failing to find an association (Kraemer et al., 2001). Evidence supporting the relationship between DHEA and waist-hip ratio is sparse. The current findings appear to suggest that DHEA increases with increases in waist-hip ratio. However, the sample for DHEA assessment was obtained upon waking. DHEA has been shown to exhibit a diurnal profile that is similar to

cortisol (Goodyer et al., 1996) with elevated DHEA in the AM phase relative to during the afternoon or evening. With this in mind, it may be that the AM sample obtained is insufficient to determine the relationship between DHEA and central obesity. Further within day assessment is therefore required.

### **5.5.2.3 Cortisol & Perceived Stress/Daily Hassles**

#### **- Perceived Stress**

Females reported greater subjective perceived stress using the Perceived Stress Scale compared with males. This is consistent with previous research in that females subjectively report more stress than males and report more physical and psychological symptoms which could indicate greater vulnerability to stress (Bebbington, 1996; Kessler et al., 1981; Kessler and McLeod, 1984; Kroenke and Spitzer, 1998; Miller and Kirsch, 1987; Troisi, 2001).

Previous research has identified a positive association between perceived stress and cortisol activity. Goldman et al. (2005), after adjusting for age and gender, found a positive association between perceived stress and cortisol. These findings were stronger in females than in males. However, Van Eck et al. (1996) failed to find an association between perceived stress and cortisol responses. In conclusion, Van Eck et al. (1996) suggested that perceived stress might not be a strong enough indicator of cortisol activity. In the current study, no association between stress (cortisol) and perceived stress using the perceived stress scale was observed.

#### **- Daily Hassles**

The subjective reporting of the intensity of the daily hassles encountered (using the Daily Hassles Scale) across the three days of sampling was significantly associated with mean cortisol. This indicated that an increase in the perceived intensity of daily hassles resulted in a reduction in mean cortisol. The subjective frequency of the daily hassles was not associated with cortisol. The association between daily hassles and cortisol is supported by previous literature. Ockenfels et al. (1995) found that experiences of stressful events (daily stressors recorded over a two consecutive day period) were associated with increases in salivary cortisol. Van Eck et al. (1996) found similar results. However, these findings did not differentiate between the number of stressor experienced and subjective intensity. Further, in the current study, the findings

suggested a reduction in cortisol activity with increasing intensity of daily hassles, conflicting with previous findings. It is possible that exposure to chronic stress in the form of daily hassles over time can cause a dysregulation of the HPA axis as in those exhibiting central obesity. In order to fully explore this hypothesis, further measures of cortisol, for example, nocturnal cortisol, are required.

The magnitude (intensity) of the stressful event is of importance, in terms of how stressful such events are perceived to be. The influence of daily hassles on subsequent cortisol responses is of greater importance when exploring the onset of depression. Sher (2004) proposed that increased experience of daily hassles and elevations in cortisol can be a characteristic of depressive behaviour and may be implicated in the pathogenesis of depression. Ultimately, the response to a stressor depends on psychological appraisal of the stressor. The reported severity of the stressful experience will reduce if the individual implements effective coping strategies. Consequently, little or no physiological stress response should be observed if coping is effective (Holroyd and Lazarus, 1982; Vogel, 1985). Similarly, if no stress responses/cortisol elevations are observed then it could be argued that the individual is demonstrating effective coping (Levine, 1978). This may contribute to the observed relationship between cortisol and subjective reporting of intensity daily hassles observed in the current study.

### **5.5.3 Classic & Non-Classic Cortisol Diurnal Profiles**

Prior to the analysis, each set of profiles was assessed individually in terms of the quality of the profile by three independent observers, who were blind to the individual characteristics of the volunteer to whom the profile belonged. Profiles were segregated into those clearly reflecting the 'classic' diurnal profile and those which did not, 'non-classic' profiles. The aim was to explore the prevalence of the 'classic' diurnal profile among a random healthy sample. A further aim was to examine the 'non-classic' profile in terms of specific characteristics that might be associated with deviance from the 'classic' profile.

#### **5.5.3.1 Metabolic Syndrome & the Basal Cortisol Profile**

An initial hypothesis proposed that those who exhibit a non-classic diurnal profile were also those individuals who exhibit central obesity; this was not found to be true. Waist-hip ratio in the classic profile group did not significantly differ from waist-hip ratio in



the non-classic profile group. Mean cortisol secreted during the monitoring period differed significantly between groups, with a greater mean of cortisol secreted in the non-classic profile (due to a blunted awakening response in conjunction with elevated evening cortisol concentrations) but this was not associated with the presence of central obesity. A second proposal, based on the theory of the metabolic syndrome as a neuroendocrine disorder (Bjorntorp and Rosmond, 2000) suggested that those who exhibit 'pathological' basal cortisol secretion are those who are most likely to also exhibit central obesity and presence of the metabolic syndrome. Bjorntorp and Rosmond (2000) assessed basal cortisol data and distinguished between two profile types, i. high morning and low evening and ii. a flat profile with lower morning values but little difference in evening values. Those who exhibited the latter profile also exhibited metabolic symptomology, endocrine abnormalities and central obesity. In the current study, insulin, cholesterol, LDL, triglycerides, IL-6, CRP and the calculated degree of insulin resistance were higher in the non-classic profile group compared with the classic profile group, but only measured level of triglycerides and CRP differed significantly. The evidence to suggest that a more disrupted or flattened profile is associated with the metabolic syndrome is therefore not strong but is suggestive as the findings point in the same direction. In the current study, the profiles were segregated purely on visual assessment to measure differences in endocrine and metabolic output. Bjorntorp and Rosmond (2000) applied a statistical weighting procedure to subgroup profiles, based on the level of variability within the profile (Rosmond et al., 1998). The resultant profiles were visually similar to the profiles obtained in the current study but the associated metabolic and endocrine characteristics were not so evident. With a statistical segregation, those differences may become more distinct.

The minimal evidence in the current study, to suggest that the difference in profiles was due to the metabolic and endocrine variation prompted the examination of other variables that could be associated with a disrupted cortisol profile. Subsequent analysis revealed that individuals who exhibited a non-classic diurnal profile also reported greater sleep disturbance, generally poor quality sleep and greater intensity of daily hassles. These are now discussed.

### **5.5.3.2 Influence of Sleep on the Basal Cortisol Profile**

The findings of the current study suggested that non-classic profiles were associated with poorer subjective sleep quality (based on subjective sleep reports from items on the LSEQ). In the 'non-classic' profile, subjective reporting of sleep disturbance was significantly greater than in the 'classic' diurnal profile. Individuals displaying a 'non-classic' profile reported more difficulty falling asleep, more periods of wakefulness and more difficulty awakening than those in the 'classic' profile group. Further, sleep quality was significantly associated with cortisol secreted during the cortisol awakening response, particularly for ease of getting to sleep, restfulness of sleep, time taken to fall asleep and ease of waking. Thus, poorer sleep quality was associated with a reduced awakening response. The non-classic profile in the current study was characterised by a lack of, or flattened, cortisol awakening response and could be explained by the high prevalence of sleep disturbance reported by individuals in this profile group. Sleep quality has been linked to changes in the cortisol awakening response. Poor sleep quality has been shown to result in a blunted awakening response (Backhaus et al., 2004; Leproult et al., 1997; Williams et al., 2005).

### **5.5.3.3 Influence of Daily Hassles on the Basal Cortisol Profile**

The findings of the current study suggested that non-classic profiles were associated with greater subjective intensity of daily hassles experienced. However, the frequency of occurrence of hassles experienced was not greater. The potential for daily hassles to influence cortisol activity is supported by previous literature. Further, the potential for daily stressors to alter HPA regulation has been raised, with observed flattened profiles in those with repeated daily stress (Stone et al., 2001). Further, Ockenfels (1995) found that altered diurnal profiles were associated with chronic stress (as marked by unemployment stress) which although the nature of the stress was different (i.e. the current study was not a study of unemployed individuals) is consistent with the findings in the current study.

### **5.5.3.4 Influence of Waking Time on the Basal Cortisol Profile**

The differences between the classic and non-classic profile groups were not attributable to differences in waking time (early/late risers). However, waking time was found to have a substantial influence on the cortisol basal diurnal profile. Early risers exhibited a significantly greater response to waking, (evidenced by a greater mean increase, AURC

and Change 0-30) and greater mean cortisol secretion during the diurnal period (Diurnal Mean). These observations are consistent with previous findings. An earlier waking time was associated with elevated cortisol across the diurnal period compared with those waking later in the day, which is consistent with findings in previous literature (Bailey and Heitkemper, 1991; Edwards et al., 2001; Kudielka and Kirschbaum, 2003; Federenko et al., 2004).

The relationship between waking time and cortisol is postulated to be related to the influence of the Suprachiasmatic nucleus (SCN) on sleep-wake cycles. Cortisol displays a consistent circadian rhythm, in part, under the control of the SCN which is associated with the control of sleep/waking cycles as previously discussed in Chapter One (Dijkswa et al., 1996; Van Cauter and Turek, 1995). During slow wave nocturnal sleep, cortisol activity is at its lowest and most stable as are levels of ACTH (Born and Fehm, 1998). Both cortisol and ACTH rise simultaneously during final stage REM sleep to produce spontaneous waking (Born et al., 1999), closely followed by the cortisol awakening response. As cortisol activity is closely linked to patterns of sleep, a wealth of research exists in support of the effect of waking time and sleep quality on subsequent cortisol responses (Bailey and Heitkemper, 1991; Edwards et al., 2001; Kudielka and Kirschbaum, 2003; Federenko et al., 2004).

Sleep duration has been shown to influence cortisol responses to waking. Shorter sleep duration can result in an elevated cortisol response on the subsequent day (Kumari et al., 2006; Leproult et al., 1997; Spath-Schwalbe et al., 1992; Wüst et al., 2000b). Unfortunately, sleep duration was not assessed in the current study. The time of waking was noted, but the time of retiring to bed on the previous evening was not. It would be easy to assume that early risers experience shorter sleep duration than late risers, hence the elevated cortisol awakening response as previously observed (Leproult et al., 1997; Spath-Schwalbe et al., 1992; Wuest et al., 2000b) but without the data this cannot be ascertained. Similarly, it could be argued that disturbed sleep and poorer sleep quality would result in shorter sleep duration with more periods of wakefulness.

It is clear that cortisol is closely associated with pattern of sleeping and sleep related behaviour, most likely mediated by an interaction between the SCN and HPA axis (Van Cauter and Turek, 1995). There have been clear effects of sleep quality and waking time

on the cortisol profiles in the current study which were compatible with the literature. The study highlights, however, the need to examine in detail the nature, timing and duration of sleep in future studies.

#### **5.5.4 Methodological Issues & Study Limitations**

##### **5.5.4.1 Compliance & the Basal Diurnal Profile**

The issue of compliance is still very important in the discussion of the current study. The inclusion of individuals who did not display a 'classic' diurnal profile (elevation after waking and lower evening cortisol) may reflect the erroneous acceptance of non-compliant volunteers. It is possible that the blunted awakening response observed in the non-classic diurnal profile group is a result of failure to adhere to the strict sampling protocol which could be manifested as disrupted cortisol activity. The cleaning and screening of data in previous studies is likely to have excluded such subjects on this basis, on the stance that it is not possible to determine whether the observed differences are due to an underlying pathological condition or simply to non-compliance. However, previous studies have also commented on the possibility that by excluding such volunteers, relevant data on those who may well be exhibiting those characteristics the research aims to examine being lost (Rivera and Svec, 1989). This will be discussed in more detail in the following section.

In terms of data screening, previous studies have recommended that subjects be excluded should no awakening response be observed in the profile (e.g. Clow et al., 2004). This is as a caution against volunteer non-compliance. However, it has been noted that approximately 10% of individuals fail to exhibit a cortisol awakening response (Prüssner et al., 1997) and indeed, this may not necessarily reflect non-compliant volunteers but leads to the exclusion of volunteers exhibiting a disrupted HPA axis. Hence, this form of exclusion was not applied in the current study. Similarly, the exclusion of individuals currently using any form of prescribed medication also excludes a potentially large number of volunteers who may have insulin resistance, diabetes or impaired glucose tolerance who would be of relevance to the aim of the current study. Such groups are excluded on the basis of these possible confounds but these individuals may be most likely to exhibit central obesity and therefore be vulnerable to differences in cortisol. Indeed Rivera and Svec (1989) argue that this is the subgroup of most interest to this hypothesis and argue further that exclusion of such

individuals could skew the characteristics of the group towards those with lower body proportions, or peripheral or whole body obesity rather than central obesity. Hence, while individuals were not excluded on the basis of their cortisol profile, the possibility, that certain medications can influence the activity of cortisol was acknowledged and individuals on prescribed medication were excluded in the current study.

In the current study, if the strategy outlined previously was applied, then a significant proportion of subjects would have been excluded from the analysis. Those exhibiting a 'non-classic' profile accounted for 48% of the whole sample, and just over half of volunteers exhibited a classic profile (52%). Should the aforementioned rule of exclusion have been applied in the current study, 48% of the sample would have been excluded. The prevalence of this 'non-classic' profile and what it could or should be attributed to require further investigation concurrent with stricter measures of compliance. For example, Kudielka et al. (2003) successfully utilised electronic monitoring devices to ensure that accurate sample timings were obtained to preserve the accuracy of data. This results in some loss of data due to incorrect sampling but a cleaner data set. However, the issue of compliance in free-living conditions is critical to the trade-off between data quality and accuracy and exclusion is difficult to resolve.

## **5.6 Conclusions**

No difference in the basal cortisol profile was observed between those with central obesity and those without. However, despite similar cortisol profiles, high waist-hip ratio individuals (centrally obese) demonstrated less mean cortisol across the profile compared with low waist-hip ratio individuals. Further, those with central obesity exhibited elevated obesity related biomarkers as expected (greater insulin and calculated degree of insulin resistance, elevated triglycerides, C - reactive protein and DHEA) when compared with those without central obesity.

Classic and non-classic cortisol profiles differed on a number of psychological and metabolic parameters. Non-classic diurnal profiles were associated with poorer sleep quality and greater subjective intensity of daily hassles. Further, non-classic profiles were associated with greater insulin resistance, elevated triglycerides and inflammatory markers, possibly signalling a vulnerability to the metabolic syndrome. This evidence seems to suggest that a culmination of contributing variables can account for differences

in the shape and magnitude of the basal cortisol profile. Poor sleeping patterns and increased intensity of daily stressors are associated with disrupted profiles and may present a vulnerability to associated metabolic symptoms which are minimal in the present sample. These may elevate with time and more chronic exposure to stress.

---

---

**CHAPTER SIX****Exploring Stress Responsivity in the Centrally Obese Using the Trier Social Stress****Test (TSST): Impact on Cognitive Function using CANTAB.**

---

---

**6.1 Introduction**

Chapter Five of this thesis explored the robustness of the basal cortisol profile in a sample of older adults. The findings suggested that subtle changes occur in the absolute level of cortisol secreted in those with central obesity compared with lean individuals with no change to the profile shape. This is consistent with previous literature, which suggests that cortisol is elevated in the centrally obese but that this elevation is not reflected in the diurnal profile. Observed cortisol concentrations are lower than in lean individuals or those with peripheral obesity, which may be attributed to an enhanced clearance rate of cortisol (Ljung et al., 1996; Marin et al., 1992; Strain et al., 1980).

The identification of alterations in basal cortisol profiles in centrally obese individuals led to the question of whether cortisol secretions in response to stress might also be different to lean individuals. Research reviewed in Chapter One suggested that cortisol responses to a psychological stressor are elevated in the centrally obese (Epel et al., 2000; Marin et al., 1992; Moyer et al., 1994) compared with lean (Moyer et al., 1994; Marin et al., 1992), peripherally obese and non-obese individuals (Epel et al., 2000).

Corticosteroid treatment has significant side effects in relation to mood and cognition (Clark et al., 1952). Research has explored the relationship between glucocorticoid activity and specific regions of the brain, which might have implications for mood and cognition. The identification of distinctive MR (mineralcorticoid) and GR (glucocorticoid) receptors (De Kloet and Reul, 1987; McEwen et al., 1986; Reul and De Kloet, 1985) within the hippocampus lent support to the observation that stress related decrements occurred in hippocampal related processing, particularly declarative memory (Lupien et al., 1994; 1997; 1998; 2005). Elevated basal cortisol in elderly adults is associated with cognitive impairments (Lupien et al., 1994; 1998). In adults,

glucocorticoid administration endogenously (e.g. Domes et al., 2002; Lupien et al., 2005) or exogenously (e.g. Wolf et al., 2001), has demonstrated that elevated cortisol levels result in impairments in the conscious retrieval of recently acquired information during both chronic and acute stress exposure (Lupien et al., 2005).

Basal cortisol activity is altered in the centrally obese and responses to stress may be more pronounced compared to non-centrally obese individuals. Cognitive decrements have been observed in the centrally obese who also show insulin resistance, high blood pressure and other features of the metabolic syndrome (Convit, 2005; Elias et al., 2005; Hashizume et al., 2006). The effect of psychological stress on cognitive function is the aim of the study presented in this chapter.

## **6.2 Objectives**

This study examined response to stress in a sample of male and female older adults aged 35-65 years during stress exposure using the Trier Social Stress Test compared with a non-stress condition. The study aimed to explore differences in stress responsivity between those exhibiting central obesity compared with lean or peripherally obese individuals (high versus low waist-hip ratio) when exposed to stress. In particular, the cortisol response to stress was examined. The influence of this on subsequent cognitive performance was investigated. Research, which specifically links cognitive performance in the centrally obese to cortisol responses, is sparse and at the time of writing represents a novel avenue for investigation.

In an attempt to replicate findings from Chapter Five, basal cortisol activity in the centrally obese was also assessed over the course of one day, prior to the test session. Associated metabolic syndrome biomarkers were assessed concurrently.

## **6.3 Method**

### **6.3.1 Sample**

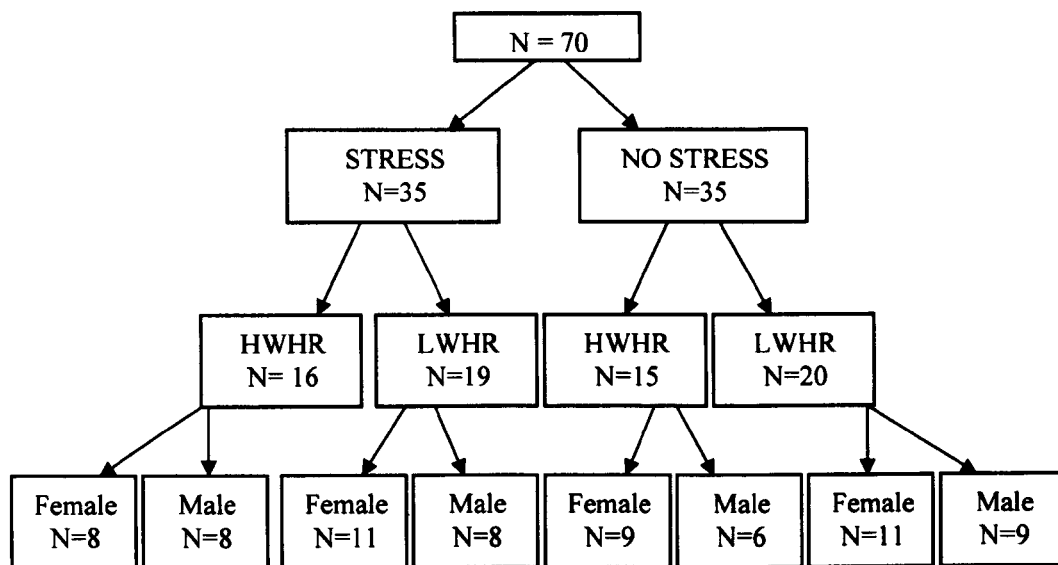
Volunteers were recruited using the same methods as outlined in Chapter Five (See Section 5.3.1). Seventy volunteers were recruited to take part in this study (See Figure 6.1) All volunteers were aged between 35 and 60 years with an average age of 46 years



$\pm 7.36$  (SD). The sample comprised 39 females (22 low waist-hip ratio and 17 high waist-hip ratio) and 31 males (17 low waist-hip ratio and 14 high waist-hip ratio). 42 volunteers from the cortisol baseline study (Chapter Five) returned to take part in this study. These were evenly distributed across the sample subgroups (Figure 6.1). The volunteers were randomly assigned to either a stress condition (the Trier Social Stress Test) or a no stress condition with 35 volunteers per condition (Figure 6.1). The same exclusion criteria as in Chapter Five (See Section 5.3.1) were applied with an additional exclusion of individuals with colour blindness (See Appendix 7).

### 6.3.2 Design

This study conformed to a 2x2x2 between subjects' analysis of variance design with 2 conditions (stress/no stress), 2 waist-hip ratio groups (high/low) and gender (male/female). This design is illustrated in Figure 6.1 below.



**Figure 6.1:** Flow Chart to illustrate the main study design WHR & gender as between subjects factors

### 6.3.3 Measures

#### 6.3.3.1 Psychological Measures

The same psychological measures were implemented as in Chapter Five. These included; i. The Hospital Anxiety and Depression Scale (HADS, Snaith and Zigmond, 1994); ii. The Dutch Eating Behaviour Questionnaire (DEBQ, Van Strien et al., 1986); iii. Perceived Stress Scale (PSS, Cohen, 1994). The National Adult Reading Test (NART; Nelson, 1982) was also administered to give a proxy measure of IQ. For a

detailed discussion of these measures, see Chapter Three. In addition, the following measures were administered:

**(i) State Trait Anxiety Inventory (STAI; Spielberger 1979)**

Since its inception, the STAI (Form X) (see Appendix 18) has been used more frequently in psychological research than any other anxiety inventory (Buros, 1978). The scale was originally developed in 1970 by Spielberger, Gorsuch and Lushene to provide an operational measure of state and trait anxiety (Vagg et al., 1980). A revised version of form X resulted in the development of the STAI Form Y which was implemented in this research to discriminate more clearly between feelings of anxiety and depression, to remove weaker psychometric properties and to improve the factor structure (Spielberger, 1983). Responses are made on a four-point scale (not at all, somewhat, moderately so and very much so). Test-retest reliability is moderate for an overall score ( $r = 0.54$ ; NHMHRDP, 1999), with good internal consistency (Cronbach alpha  $\alpha = 0.83-0.93$ ; Hishinuma et al., 2000). The STAI was implemented to measure changes in state anxiety during the experimental test session.

**(ii) State Self Esteem Scale (SSES; Heatherton and Polivy, 1991)**

The State Self-Esteem Scale (SSES) (see Appendix 19) was developed as a measure of short-lived changes in self-esteem (Heatherton and Polivy 1991). The SSES consists of 20 items adapted from the Janis-Field Feelings of Inadequacy Scale (Janis and Field, 1959). There are three esteem factors in the scale (i) academic performance (ii) social evaluation and (iii) appearance. The internal consistency of the scale is good ( $\alpha = 0.92$ ). The SSES; was implemented to measure changes in state self-esteem during the experimental test session.

**6.3.3.2 Biological Measures**

At screening, i. blood pressure, ii. body mass index, iii. body composition, iv. waist-hip ratio and a range of blood biomarkers (glucose, insulin, calculated degree of insulin resistance, total cholesterol, HDL, LDL, triglycerides, IL-6, CRP and adiponectin) were obtained (See Chapter Three, Section 3.2.3.1).

During the study period, measures of salivary cortisol, blood pressure and dehydroepiandrosterone (DHEA) were obtained (See Chapter Three, Sections 3.3 and 3.4.3.1).

#### **6.3.4 Procedure**

Following telephone screening and completion of the Volunteer Screening Booklet (See Appendix 1), screened volunteers were asked to attend an induction session at the Institute of Psychological Sciences. This session introduced the volunteer to the study, the equipment and procedure. Volunteers were told that they were to complete a number of mentally challenging psychological tests to assess cognitive ability. Prior to study commencement, ethical approval for the study was obtained from the Institute of Psychological Sciences Ethics Committee. Reading ability as a proxy measure of IQ using the NART and colour blindness were assessed.

##### **6.3.4.1 Monitoring Day One**

The monitoring day was the day immediately prior to the test day. Volunteers followed the same procedure as detailed in Chapter Five (See Section 5.3.4.1) (see Appendices 3 and 4).

##### **6.3.4.2 Test Day**

On the test day morning, volunteers followed the same procedure as in Chapter Five for the collection of saliva to determine the cortisol awakening response.

Following the final saliva sample, for determination of the cortisol awakening response at 45minutes post waking, volunteers were permitted to consume breakfast, which they were advised should be something that they would normally consume. Post breakfast, volunteers were asked to refrain from snacks until their appointment at the Institute when a lunch was provided. Lunch consisted of a white bread cheese sandwich with salted crisps. This ad libitum meal ensured that all volunteers had eaten to a level of comfortable fullness prior to the test session. Volunteers were asked to refrain from any strenuous physical activity for at least one hour prior to the experiment. Appointments for the test session were consistently scheduled for 1.30pm on each test day.

A summary of the test session procedure is detailed in Figure 6.2 below.

Time	STRESS (S)	NO STRESS (NS)
1.30	Standard lunch	
1.50	Baseline measures (BP, Cortisol, STAI, SSES)	
2.00	Meet panel	Shown job advert
	10mins preparation	
	BP, Cortisol, STAI, SSES measured	
2.10	TSST public speaking task	Friendly discussion of advert
	BP and Cortisol measured	
	TSST mental arithmetic	Basic arithmetic worksheet
2.20	BP, Cortisol, STAI, SSES measured	
2.30	Cognitive test battery	
3.30	BP, Cortisol, STAI, SSES measured	
3.40	Cortisol sample and BP measured. Study terminated (debrief questionnaire and information sheet provided)	

**Figure 6.2:** Test Session Schedule of Events

- i. Upon arrival, the volunteer was provided with lunch and allowed to relax. Twenty minutes post arrival, baseline measures of salivary cortisol and BP were collected. The STAI and SSES questionnaires were completed (Figure 6.1).
- ii. The volunteer was subsequently exposed to either the stress (TSST) or no-stressor task depending on randomisation (see Figure 6.1). The tasks are explained in detail in Chapter Three).
- iii. Following the preparation phase of the task, a cortisol saliva sample was obtained with measurement of blood pressure and completion of the STAI and SSES questionnaires (Figure 6.1).
- iv. Following the ‘presentation’(S)/‘chat’ (NS) phase of the task, a cortisol saliva sample was collected with measurement of blood pressure. The mental arithmetic phase of the task was subsequently completed (Figure 6.1).
- v. Following completion of the stress/no stressor task, the volunteer provided a cortisol saliva sample, measurement of blood pressure was recorded and the STAI and SSES

questionnaires were completed. The volunteer was asked to begin the cognitive test battery (using CANTAB – Chapter Three Section 3.5). The order and timing of the battery is shown below:

i.	<b>Auditory Verbal Learning Task (AVLT) Part One:</b>	
	List A repeated five times followed by List B (distracter)	5 minutes
ii.	CANTAB: Motor Screening practice session (MOT)	2 minutes
iii.	CANTAB: Delayed Matching to Sample (DMS)	5 minutes
iv.	CANTAB: Paired Associates Learning Task (PAL)	8 minutes
v.	<b>Auditory Verbal Learning Task (AVLT) Part Two:</b>	
	Delayed Recall of List A	3 minutes
vi.	CANTAB: Pattern Recognition Memory (PRM)	5 minutes
vi.	CANTAB: Rapid Visual Processing (RVP)	5 minutes
viii.	CANTAB: Spatial Recognition Memory (SRM)	5 minutes
ix.	CANTAB: Spatial Working Memory	10 minutes
x.	CANTAB: Stockings of Cambridge (SOC)	8 minutes
	<b>Total Time:</b>	<b>56 minutes</b>

viii. Upon completion of the cognitive test battery, the volunteer provided a cortisol saliva sample with measurement of blood pressure and completion of the STAI and SSES. The volunteer was asked to rest.

ix. Following 5-minutes of relaxation a final cortisol saliva was collected. The experiment was terminated. A debrief questionnaire was administered to assess the individual's perception of how stressful the Trier Social Stress Test or the no stressor task was perceived to be and to rate their subjective performance on the cognitive test battery (See Appendix 8 and 9).

#### **6.3.4.3 Monitoring Day Two**

Volunteers were required to provide saliva samples using salivettes for cortisol analysis from waking on the day post-test session completion to assess the CAR and to examine the influence of stress exposure on subsequent cortisol responses to waking. The same procedure was used for monitoring day one (See Chapter Five, Section 5.3.4.1). However, samples were only obtained on waking until 45minutes post waking.

Collected saliva samples were refrigerated until returned to the Institute where they were subsequently frozen at -20°C.

### **6.3.5 Data Treatment and Analysis**

#### **6.3.5.1 Missing Data**

As in Chapter Five, this study relies upon the compliance of the volunteer to produce the required samples at the specified times. In addition, adequate volumes of saliva were required for assay. During the test session, the timing of the samples was determined by the presence of the researcher. However, adequate volumes of saliva were not always obtained. This was of particular relevance during the TSST when a dry mouth (due to the public speaking task and a highly stressful situation) was common.

This analysis of cortisol (both basal and in response to stress) did not include the full sample (n=70) because of missing data or lack of sufficient saliva. The data for the blood biomarkers were almost complete with only one exclusion due to an unsuccessful venepuncture. The sample size ranged from 65 to 69 within the analysis for each hypothesis. For analysis of cognitive performance, subjective measures of mood, well-being and performance and blood pressure complete data were obtained.

#### **6.3.5.2 Data Screening**

All data was screened for outliers using boxplots conducted in SPSS prior to analysis. There were few outliers so the analysis was subsequently conducted with outliers included unless they were extreme outliers, in which case the analysis was re-run with the extreme outliers excluded. All data was explored using SPSS and normality assessed using histograms and the Kolmogorov-Smirnov test of normality. The cortisol data was found to be positively skewed across all sample points and was normalised using a logarithmic transformation. All analyses were conducted using SPSS version 12.

#### **6.3.5.3 Statistical Analysis**

##### **(i) Stress Responsivity, Central Obesity and Cognitive Performance**

Cortisol responses obtained over the duration of the test session were analysed using a 2x2x2x6 repeated measures analysis of variance model with condition (stress/no stress), waist-hip ratio (high/low) and gender (male/female) as between subjects factors and time as a within subjects factor. As cortisol responses vary with age, age was included

as a covariate. Significant interactions were explored post hoc using Bonferroni corrected independent samples t-tests. Cortisol response to the stress/no stress task was also assessed using change from baseline (sample 1 to the mean of time points 3 and 4). This was calculated as percentage increase and analysed using a 2x2x2 between subjects ANOVA. Significant interactions were explored post hoc using Bonferroni corrected independent samples t-tests. The same analysis was conducted to assess recovery using change from baseline to the final sample as the dependent variable. Blood pressure and changes in mood/well-being using the STAI and SSES were analysed in the same way as for salivary cortisol.

Scores generated from the CANTAB data handling facility were imported and analysed in SPSS. Performance on each task was analysed individually using a 2x2x2 (waist-hip ratio, gender, condition) between subjects ANOVA with age and predicted IQ (from the NART) as covariates. Scores from the AVLT were entered manually for each of the five trials and analysed using SPSS. Where multiple trials were performed, trial was included as a within subjects factor. The number of words recalled in each trial relative to baseline was analysed using the same procedure. The total number of words recalled across the five trials, number of words recalled on list B and the delayed recall of list A were assessed. Finally, pro-active interference was assessed using a calculation of the difference between the number of words recalled in list A trial one and in List B. This assessed the ability of previously learned material to influence acquisition of new material. Cognitive performance on each task was analysed according to (i) condition (stress/no stress), (ii) actual cortisol response (cortisol responders/non-responders) and (iii) overall stress response based on both cortisol and blood pressure responses (combined BP and cortisol responders/non-responders) using 2x2x2 between subjects ANOVA's.

### **(ii) Associated Psychological and Biological Parameters**

In an attempt to replicate findings from Chapter Five, a basic analysis of the basal diurnal profile was conducted. A repeated measures ANOVA assessed cortisol profiles according to waist-hip ratio and gender (between subjects' factors). Age was included as a covariate. Significant interactions were explored post hoc using Bonferroni corrected independent samples t-tests. The cortisol awakening response and subsequent diurnal activity on monitoring day one using the calculated indices of cortisol activity

(see Chapter Three Section 3.3.3) were analysed individually using univariate ANOVA's. Further, a blind categorisation of the basal cortisol profiles was conducted using the same procedure as outlined in Chapter Five (Section 5.2.5.1). The profiles were subsequently compared in terms of associated psychological and metabolic parameters using a 2x2x2 ANOVA including profile group as a between subjects factor. Age was included as a covariate.

Subjective performance and blood biomarkers were analysed using analysis of variance with condition, waist-hip ratio and gender as between subjects' factors. Analysis of blood biomarkers included mean cortisol and AUC index of awakening response activity on the monitoring day as covariates. DHEA was analysed using a repeated measures ANOVA with waist-hip ratio and gender as between subjects' factors and day (Monitoring Day One, Test Day, Monitoring Day Two) as a within subjects factor. Age was included as a covariate. Covariates were assessed in terms of the strength of their relationship with each dependent variable using separate linear regression analyses and beta values were reported.

## **6.4 Results**

### **6.4.1 Sample Characteristics**

A summary of the main characteristics of the sample are shown in Table 6.1. A trend for a waist-hip ratio group\*gender interaction for age was observed ( $F(1, 62) = 3.884$ ;  $p = 0.053$ ). Post hoc analysis revealed that low waist-hip ratio males were, on average, the youngest members of the sample being significantly younger than low waist-hip ratio females ( $p = 0.040$ ) and high waist-hip ratio males ( $p = 0.023$ ). A significant main effect of waist-hip ratio on perceived stress score was observed ( $F(1, 62) = 4.870$ ;  $p < 0.05$ ). Those with central obesity (HWHR) reported greater subjective stress than the low waist-hip ratio subgroup. Measured waist-hip ratio was significantly higher in the high waist-hip ratio groups than in the low waist-hip ratio groups as expected ( $F(1, 62) = 149.33$ ;  $p < 0.01$ ). Further, a significant main effect of body mass index (BMI) was observed ( $F(1, 62) = 43.405$ ;  $p < 0.01$ ). BMI was significantly greater in the high waist-hip ratio groups compared with the low waist-hip ratio group.



Characteristic	Total N=70	HWHR		LWHR		HWHR		LWHR		HWHR		LWHR	
		Male, Stress N =8	Male, No Stress N =6	Male, Stress N =8	Male, No Stress N =9	Female, Stress N =8	Female, No Stress N =9	Female, Stress N =8	Female, No Stress N =11	Female, Stress N =9	Female, No Stress N =11	Female, Stress N =9	Female, No Stress N =11
Age (years)	46±7.36	48.75±8.01	48.33±8.57	41.50±4.38	42.89±8.62	48.88±6.31	46.55±7.17	44.00±6.89	47.55±7.39				
BMI (Kg/m <sup>2</sup> )	28±6.38	32.00±7.52	29.67±6.65	25.38±3.75	23.71±1.62	31.53±2.86	23.65±3.42	35.82±7.61	24.94±3.31				
WHR	0.86±0.09	0.96±0.05	0.95±0.06	0.84±0.03	0.85±0.02	0.91±0.05	0.76±0.05	0.91±0.03	0.76±0.04				
PSS Score	17±7.12	19.00±9.04	17.00±6.42	15.13±4.05	14.33±6.69	17.13±7.53	17.09±6.82	22.56±6.02	14.18±7.78				
Estimated IQ	119±4.39	120±4.27	122±1.94	121±3.51	120±3.02	116±6.09	120±4.02	117±4.36	117±4.59				

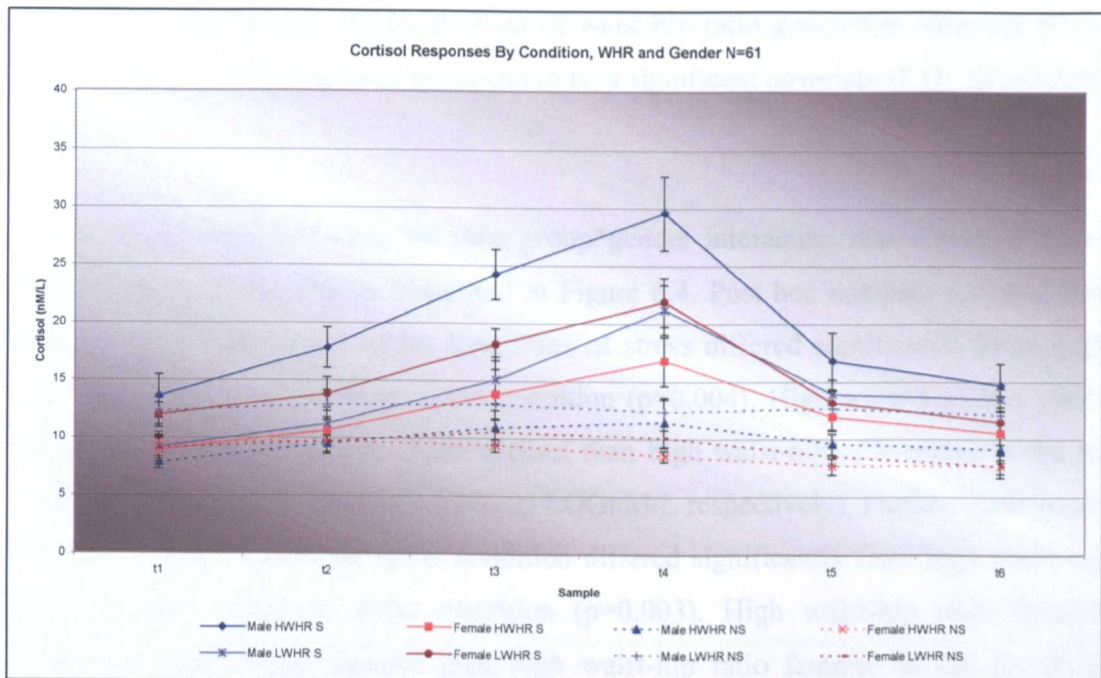
**Table 6.1:** Sample Characteristics displaying age, BMI, WHR, Estimated IQ & Perceived Stress with Means ± SD

Finally, a main effect of gender on estimated IQ (as determined by the NART) was observed ( $F(1, 62) = 141.583$ ;  $p < 0.01$ ). Estimated IQ was significantly higher in males compared with females. No other differences were observed between the respective groups.

## 6.4.2 Stress Responsivity, Central Obesity & Cognitive Performance

### 6.4.2.1 Salivary Cortisol in Response to the Stress/No Stress Tasks in High/Low WHR

Salivary cortisol responses to stress were assessed in high and low WHR males and females using a repeated measures ANOVA on the six samples taken during the stress/no stress exposure. Due to missing data, 61 observations of 70 were included in the analysis. The mean cortisol at each time point for each group is shown in Figure 6.3.



**Figure 6.3:** Cortisol secreted across the test session in High/Low WHR Males/Females (Means  $\pm$  SEM)

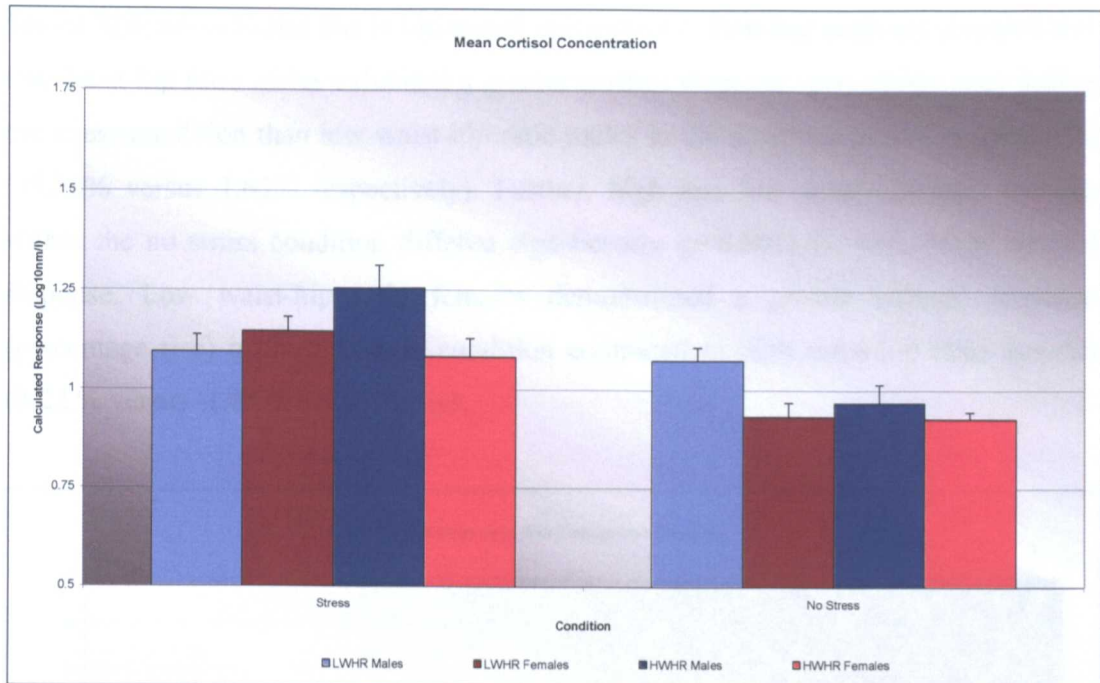
The potential four way time\*condition\*waist-hip ratio group\*gender interaction also failed to reach significance ( $F(1, 52) = 9.336$ ;  $p = 0.079$ ; NS).

A significant time\*waist-hip ratio group\*gender interaction was observed ( $F(5, 260) = 2.397$ ;  $p < 0.05$ ). However, Bonferroni corrected t-tests failed to reach significance for

any post hoc comparisons. Based on means, high waist-hip ratio males appeared to exhibit greater cortisol response at time points 3 and 4 than high waist-hip ratio females. A significant time\*condition interaction was observed ( $F(5,260) = 13.973$ ;  $p < 0.01$ ). Cortisol responses in the stress condition were significantly higher than cortisol responses in the no stress condition at time point 2 ( $p = 0.022$ ), 3 ( $p = 0.001$ ), 4 ( $p = 0.001$ ), 5 ( $p = 0.001$ ) and 6 ( $p = 0.001$ ). Baseline cortisol at time 1 did not differ significantly between conditions ( $p = 0.201$ ).

A main effect of condition for mean cortisol across the test session was observed ( $F(1, 52) = 36.558$ ;  $p < 0.01$ ). Significantly, greater mean cortisol was observed in the stress condition than in the no stress condition ( $1.44 \pm 0.02$  and  $0.975 \pm 0.02$  LOGnM/L respectively). Further, a main effect of gender was observed ( $F(1, 52) = 7.946$ ;  $p < 0.01$ ). Males exhibited greater mean concentrations than females ( $1.10 \pm 0.21$  and  $1.02 \pm 0.19$  LOGnM/L respectively). No main effect of waist-hip ratio group was observed ( $F(1, 52) = 0.138$ ;  $p = 0.712$ ). Age was not found to be a significant covariate ( $F(1, 52) = 1.195$ ;  $p = 0.279$ ; NS).

A significant condition\*waist-hip ratio group\*gender interaction was observed ( $F(1, 52) = 9.336$ ;  $p < 0.01$ ). This is illustrated in Figure 6.4. Post hoc analyses revealed that high waist-hip ratio males under conditions of stress differed significantly from high waist-hip ratio males in the no stress condition ( $p = 0.004$ ). High waist-hip ratio males under stress exhibited greater mean cortisol than high waist-hip ratio males in the no stress condition ( $1.26 \pm 0.06$  and  $0.97 \pm 0.05$  LOGnM/L respectively). Further, high waist-hip ratio females within the stress condition differed significantly from high waist-hip ratio females in the no stress condition ( $p = 0.003$ ). High waist-hip ratio females exhibited greater mean cortisol than high waist-hip ratio females in the no stress condition ( $1.08 \pm 0.05$  and  $0.93 \pm 0.02$  LOGnM/L in females). Low waist-hip ratio females in the stress condition also differed significantly from low waist-hip ratio females in the no stress condition ( $p = 0.001$ ). Females in the stress condition exhibited a greater mean of cortisol across the test session ( $1.15 \pm 0.04$  and  $0.93 \pm 0.03$  LOGnM/L respectively). There was an observed trend for high and low waist-hip ratio males in the stress condition to differ ( $p = 0.046$ ). High waist-hip ratio males secreted greater cortisol during the stress condition than low waist-hip ratio males in the same condition ( $1.26 \pm 0.06$  and  $1.10 \pm 0.04$  LOGnM/L).



**Figure 6.4:** Mean Cortisol secreted during the test session in High/Low WHR Males/Females by Stress/No Stress Condition (Means  $\pm$  SEM)

#### 6.4.2.2 Calculated Cortisol Response to the Stress/No Stress Tasks (Percentage Rise)

The percentage increase (change from baseline) from baseline to mean cortisol at time points 3 (during the task) and 4 (immediately post stress/no stress task) was calculated and analysed using a 2x2x2 ANOVA with waist-hip ratio (high/low), gender (male/female) and condition (stress/no stress) as between subjects factors. The mean responses by gender and WHR for percentage rise are shown in Table 6.2.

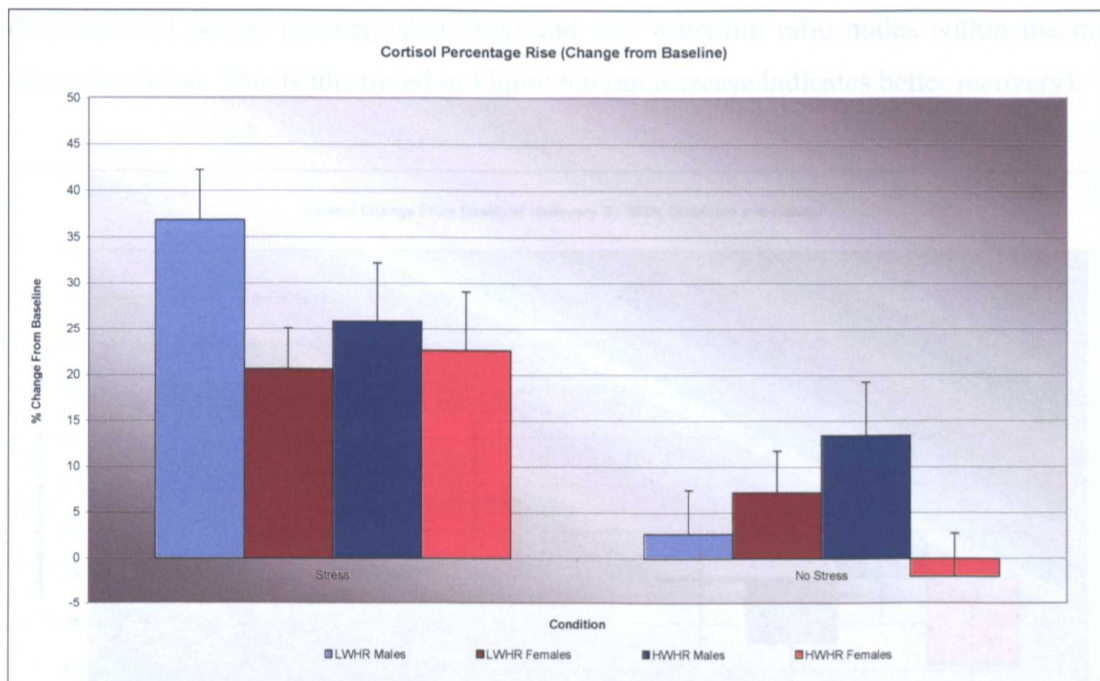
Condition (Means  $\pm$  SEM)

	Total (%)	Male HWHR%	Male LWHR%	Fem HWHR%	Fem LWHR%
Stress	26.17 $\pm$ 16.14	26.72 $\pm$ 7.55	35.58 $\pm$ 22.20	23.94 $\pm$ 13.45	20.42 $\pm$ 14.49
No Stress	4.88 $\pm$ 13.42	14.33 $\pm$ 15.96	1.63 $\pm$ 6.05	-1.86 $\pm$ 7.03	8.22 $\pm$ 17.73

**Table 6.2:** Mean Percentage Rise by WHR and gender (Mean  $\pm$  SD)

It is evident that cortisol responses were greater under stress evidenced by a significant main effect of condition ( $F(1, 52) = 31.531$ ;  $p < 0.01$ ) was observed for the percentage rise in cortisol from baseline to post stress/no stress task. A greater cortisol response (26%) was observed in the stress condition compared with the no stress condition (5%).

A significant condition\*waist-hip ratio group\*gender interaction was observed ( $F(1, 52) = 4.528$ ;  $p < 0.05$ ) and this is illustrated in Figure 6.5. Post hoc analyses revealed that low waist-hip ratio males exhibited a greater cortisol response (percentage rise) during the stress condition than low waist-hip ratio males in the no stress condition ( $p = 0.001$ ) (35.58% versus 1.63% respectively). Further, high and low waist-hip ratio females within the no stress condition differed significantly ( $p = 0.001$ ) for percentage cortisol response. Low waist-hip ratio females demonstrated a greater cortisol response (percentage rise) in the no stress condition compared to high waist-hip ratio females (8.22% versus -1.86% respectively).



**Figure 6.5:** % Rise in Cortisol in High/Low WHR Males/Females by Stress/No Stress Condition (Means  $\pm$  SEM)

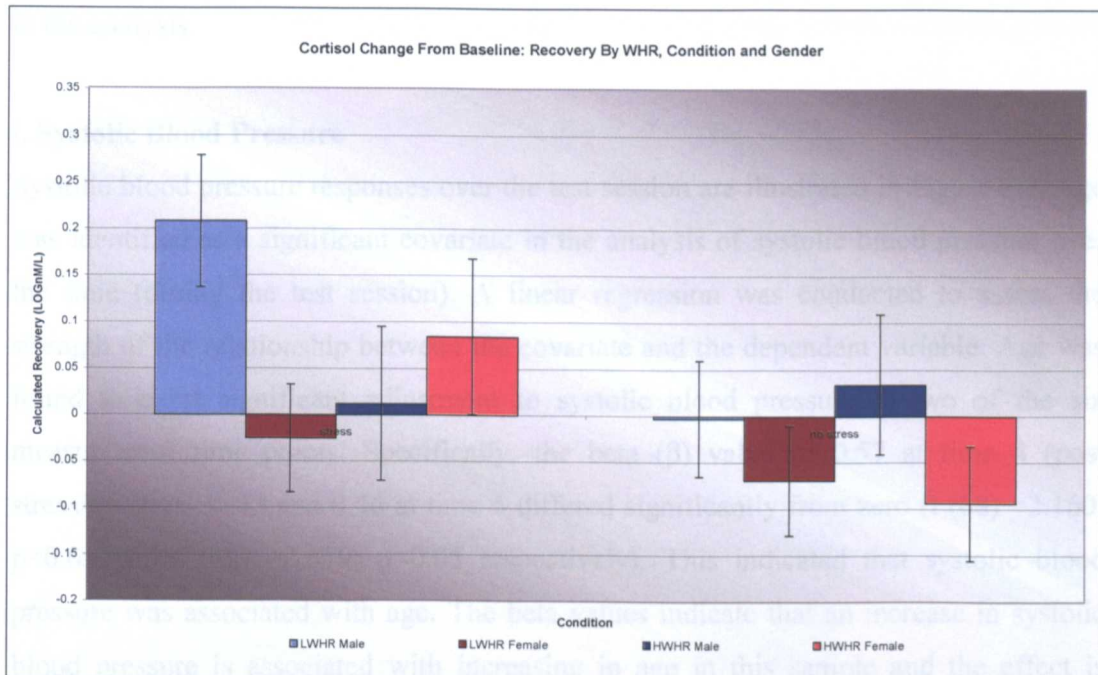
The condition\*waist-hip ratio group interaction ( $F(1, 52) = 0.554$ ;  $p = 0.460$ ; NS), condition\*gender interaction ( $F(1, 52) = 0.383$ ;  $p = 0.539$ ; NS) and waist-hip ratio\*gender interaction ( $F(1, 52) = 0.163$ ;  $p = 0.688$ ; NS) all failed to reach significance.

The condition\*waist-hip ratio group interaction ( $F(1, 52) = 0.247$ ;  $p = 0.621$ ; NS). Further, a trend for a main effect of gender was observed ( $F(1, 52) = 3.859$ ;  $p = 0.055$ ). Males were more responsive than females (showing mean increases of 19% and 12% respectively). No main effect of waist-hip ratio was observed ( $F(1, 52) = 0.182$ ;  $p = 0.671$ ; NS). Age was not found to be a significant covariate ( $F(1, 52) = 1.534$ ;  $p = 0.221$ ; NS). Individuals in the stress condition demonstrated less recovery post

### 6.4.2.3 Cortisol Change from Baseline (Recovery)

Recovery was calculated as a change from baseline to the final sample obtained (Sample 6). Recovery was the dependent variable in a 2x2x2 between subjects ANOVA with waist-hip ratio, gender and condition as between subjects' factors.

A trend for a condition\*waist-hip ratio\*gender interaction was observed ( $F(1, 52) = 3.440$ ;  $p = 0.069$ ), however, Bonferroni corrected t-tests failed to reach significance. Based on means, there was a tendency for high waist-hip ratio males to show poorer recovery than low waist-hip ratio males within the stress condition ( $0.029 \pm 0.06$  and  $0.183 \pm 0.05$  LOGnM/L respectively). Both high and low waist-hip ratio females demonstrated poorer recovery than high and low waist-hip ratio males within the no stress condition. This is illustrated in Figure 6.6 (an increase indicates better recovery).



**Figure 6.6:** Recovery of Cortisol activity in High/Low WHR Males/Females by Stress/No Stress Condition (Means  $\pm$  SEM)

The condition\*waist-hip ratio group interaction ( $F(1, 52) = 0.247$ ;  $p = 0.621$ ; NS), condition\*gender interaction ( $F(1, 52) = 0.029$ ;  $p = 0.865$ ; NS) and waist-hip ratio\*gender interaction ( $F(1, 52) = 1.478$ ;  $p = 0.230$ ; NS) failed to reach significance.

A main effect of condition on recovery post cortisol response was observed ( $F(1, 52) = 4.405$ ;  $p < 0.05$ ). Individuals in the stress condition demonstrated less recovery post

stress/no stressor task compared to individuals in the no stress condition ( $0.070 \pm 0.04$  and  $-0.033 \pm 0.03$  LOGnM/L respectively). A trend for a main effect of gender was also observed ( $F(1, 52) = 3.269$ ;  $p = 0.076$ ). Males demonstrated poorer recovery than females ( $0.063 \pm 0.04$  and  $-0.026 \pm 0.03$  LOGnM/L respectively). No main effect of waist-hip ratio group was observed ( $F(1, 52) = 0.136$ ;  $p = 0.714$ ; NS). Age was not found to be a significant covariate ( $F(1, 52) = 3.616$ ;  $p = 0.063$ ). However, the linear regression indicated that recovery worsened with age.

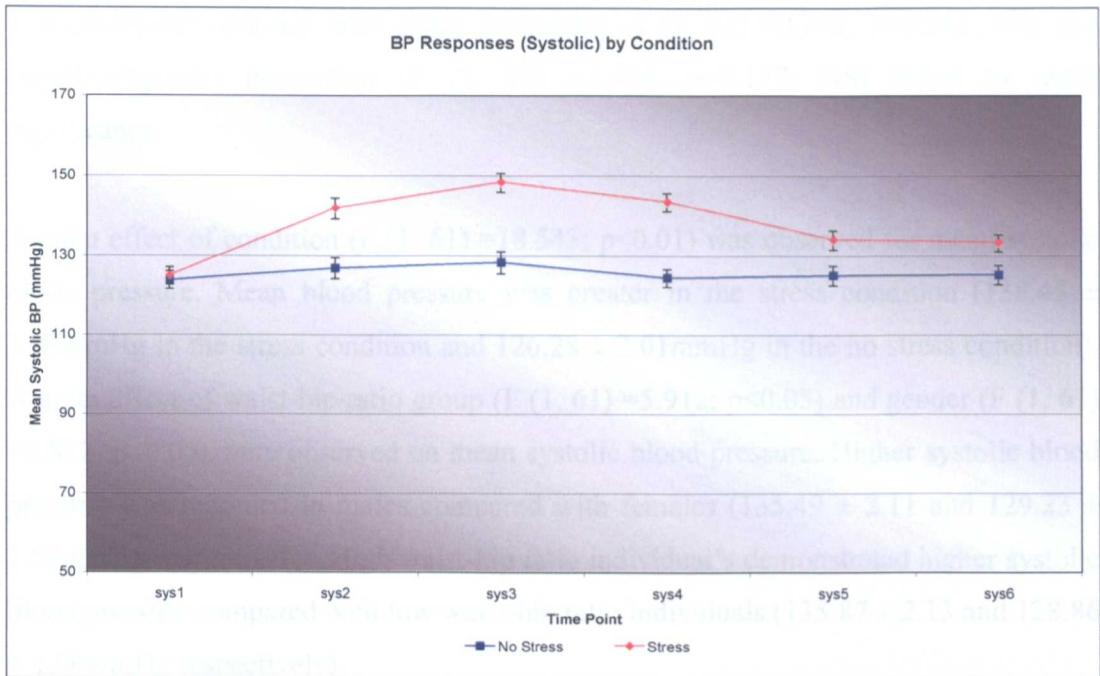
#### **6.4.2.4 Blood Pressure Response to the Stress/No Stress Tasks in High/Low WHR Males and Females**

Blood pressure responses (systolic and diastolic) were analysed over the test session using a  $2 \times 2 \times 2 \times 8$  ANOVA with condition, waist-hip ratio and gender as between subjects factors and time as a within subjects factor. All 70 observations were included in the analysis.

##### **i. Systolic Blood Pressure**

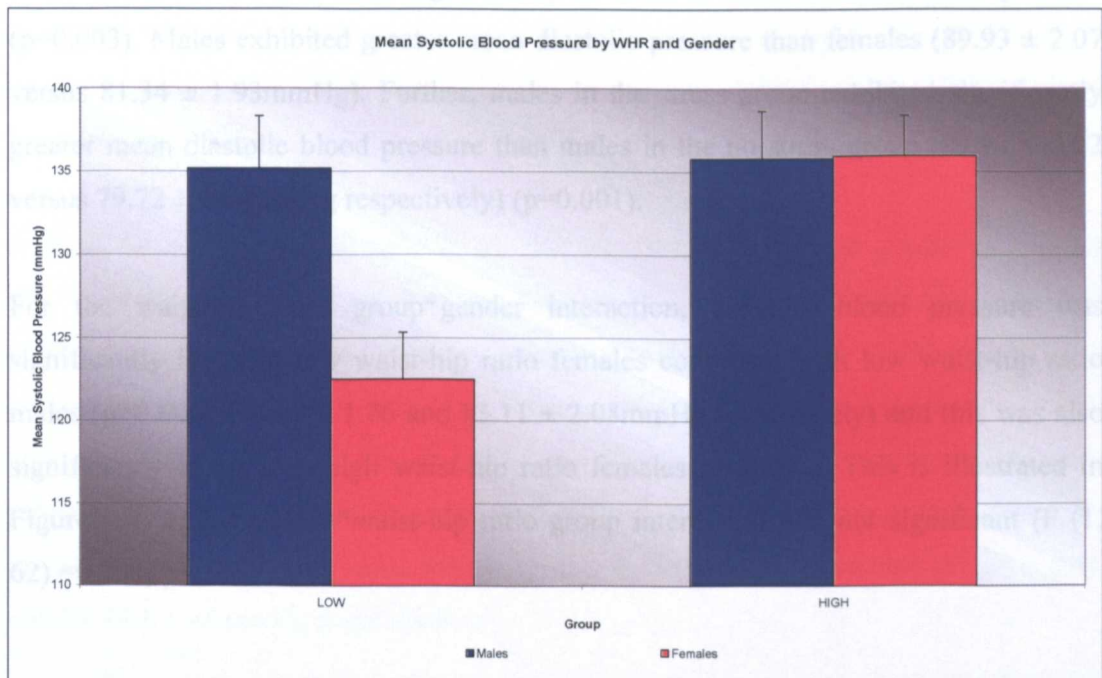
Systolic blood pressure responses over the test session are illustrated in Figure 6.7. Age was identified as a significant covariate in the analysis of systolic blood pressure over the time (during the test session). A linear regression was conducted to assess the strength of the relationship between the covariate and the dependent variable. Age was found to exert significant adjustment to systolic blood pressure at two of the six measurement time points. Specifically, the beta ( $\beta$ ) value of 0.57 at time 4 (post stress/no stress task) and 0.46 at time 6 differed significantly from zero ( $t(68) = 2.160$ ;  $p < 0.05$  and  $t(68) = 2.079$ ;  $p < 0.05$  respectively). This indicated that systolic blood pressure was associated with age. The beta values indicate that an increase in systolic blood pressure is associated with increasing in age in this sample and the effect is greatest immediately post stress, that is older individuals demonstrate the greatest increase in systolic blood pressure.

The condition\*waist-hip ratio\*gender interaction was non-significant ( $F(1, 62) = 0.238$ ;  $p = 0.627$ ; NS). A significant time\*condition interaction was observed for blood pressure measured across the session ( $F(5, 305) = 23.144$ ;  $p < 0.01$ ). Systolic blood pressure was significantly higher in the stress condition compared with the no stress condition at time points 2 ( $p = 0.001$ ), 3 ( $p = 0.001$ ) and 4 ( $p = 0.001$ ). This is illustrated in Figure 6.7.



**Figure 6.7:** Systolic Blood Pressure Responses by Stress/No Stress Condition (Means ± SEM)

A significant waist-hip ratio group\*gender interaction was observed ( $F(1, 61) = 4.880$ ;  $p < 0.05$ ). Post hoc analyses revealed that low waist-hip ratio females exhibited significantly lower mean systolic blood pressure ( $122.52 \pm 2.48$  mmHg) compared with high waist-hip ratio females ( $135.20 \pm 2.92$  mmHg) ( $p = 0.008$ ) while there was no difference in high and low waist-hip ratio males. This is illustrated in Figure 6.8 below.



**Figure 6.8:** Systolic Blood Pressure Responses in High/Low WHR Males/Females (Means ± SEM)



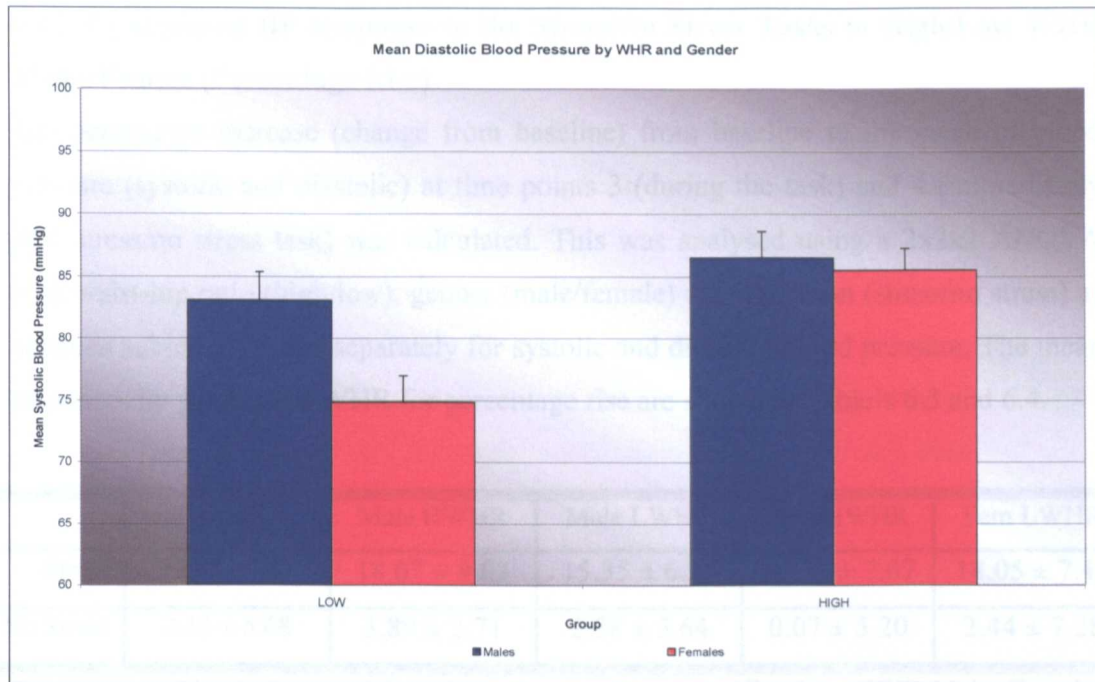
The condition\*waist-hip ratio group interaction ( $F(1, 52) = 0.378$ ;  $p = 0.541$ ; NS) and condition\*gender interaction ( $F(1, 52) = 1.864$ ;  $p = 0.177$ ; NS) failed to reach significance.

A main effect of condition ( $F(1, 61) = 18.543$ ;  $p < 0.01$ ) was observed for mean systolic blood pressure. Mean blood pressure was greater in the stress condition ( $138.45 \pm 1.98$  mmHg in the stress condition and  $126.28 \pm 2.01$  mmHg in the no stress condition). A main effect of waist-hip ratio group ( $F(1, 61) = 5.912$ ;  $p < 0.05$ ) and gender ( $F(1, 61) = 4.872$ ;  $p < 0.05$ ) were observed on mean systolic blood pressure. Higher systolic blood pressure was recorded in males compared with females ( $135.49 \pm 2.11$  and  $129.23 \pm 1.88$  mmHg respectively). High waist-hip ratio individuals demonstrated higher systolic blood pressure compared with low waist-hip ratio individuals ( $135.87 \pm 2.13$  and  $128.86 \pm 1.90$  mmHg respectively).

## ii. Diastolic Blood Pressure

The condition\*waist-hip ratio\*gender interaction was non-significant ( $F(1, 62) = 0.022$ ;  $p = 0.883$ ; NS). Trends were evident for a condition\*gender interaction ( $F(1, 62) = 3.842$ ;  $p = 0.055$ ) and the waist-hip ratio group\*gender interaction ( $F(1, 62) = 3.043$ ;  $p = 0.086$ ). For the condition\*gender interaction, post hoc analyses revealed that males and females in the stress condition differed significantly in terms of mean diastolic blood pressure ( $p = 0.003$ ). Males exhibited greater mean diastolic pressure than females ( $89.93 \pm 2.07$  versus  $81.34 \pm 1.93$  mmHg). Further, males in the stress group exhibited significantly greater mean diastolic blood pressure than males in the no stress group ( $89.93 \pm 2.22$  versus  $79.72 \pm 1.64$  mmHg respectively) ( $p = 0.001$ ).

For the waist-hip ratio group\*gender interaction, diastolic blood pressure was significantly lower in low waist-hip ratio females compared with low waist-hip ratio males ( $p = 0.005$ ) ( $74.88 \pm 1.76$  and  $83.11 \pm 2.08$  mmHg respectively) and this was also significantly lower than high waist-hip ratio females ( $p = 0.001$ ). This is illustrated in Figure 6.9. The condition\*waist-hip ratio group interaction was not significant ( $F(1, 62) = 0.151$ ;  $p = 0.699$ ; NS).



**Figure 6.9:** Diastolic Blood Pressure Responses in High/Low WHR Males/Females (Means  $\pm$  SEM)

A significant time\*condition interaction was observed ( $F(5,300) = 12.357$ ;  $p < 0.01$ ). The pattern was similar to that of systolic blood pressure shown in Figure 6.7. Post hoc analyses revealed that diastolic blood pressure was significantly higher in the stress condition at time points 2 (post preparation), 3 (during the task) and 4 (post task) compared with the no stress condition (smallest  $p = 0.001$ ). As expected, the stress and no stress conditions differed significantly in terms of overall mean diastolic blood pressure ( $p = 0.003$ ).

A main effect of waist-hip ratio ( $F(1, 60) = 11.590$ ;  $p < 0.01$ ) and gender ( $F(1, 60) = 5.146$ ;  $p < 0.05$ ) was observed for mean diastolic blood pressure. Higher mean diastolic blood pressure was recorded in males compared to females ( $84.82 \pm 1.50$  and  $80.21 \pm 1.36$  mmHg respectively) and in high waist-hip ratio individuals compared with low waist-hip ratio individuals ( $86.04 \pm 1.54$  and  $79 \pm 1.35$  mmHg respectively). A main effect of condition was also observed ( $F(1, 62) = 9.512$ ;  $p < 0.01$ ). Mean diastolic blood pressure was higher in the stress condition than in the no stress condition ( $85.64 \pm 1.41$  and  $79.40 \pm 1.45$  mmHg respectively).

#### L. Anticipation

Anticipation was calculated as the change from baseline to the blood pressure reading obtained at time 2 (post preparation and pre-stress/no stress task). Anticipation was the

#### 6.4.2.5 Calculated BP Response to the Stress/No Stress Tasks in High/Low WHR Males/Female (Percentage Rise)

The percentage increase (change from baseline) from baseline to the mean of blood pressure (systolic and diastolic) at time points 3 (during the task) and 4 (immediately post stress/no stress task) was calculated. This was analysed using a 2x2x2 ANOVA with waist-hip ratio (high/low), gender (male/female) and condition (stress/no stress) as between subjects' factors separately for systolic and diastolic blood pressure. The mean responses by gender and WHR for percentage rise are shown in Table's 6.3 and 6.4.

	Total (%)	Male HWHR	Male LWHR	Fem HWHR	Fem LWHR
Stress	17.04 ± 7.27	18.07 ± 9.03	15.35 ± 6.27	16.31 ± 7.07	18.05 ± 7.45
No Stress	2.12 ± 5.68	3.89 ± 2.71	2.58 ± 5.64	0.07 ± 5.20	2.44 ± 7.28

**Table 6.3:** % Rise in Systolic Blood Pressure post stressor in High/Low WHR Males/Females (Means ± SD)

	Total (%)	Male HWHR	Male LWHR	Fem HWHR	Fem LWHR
Stress	15.09 ± 9.09	17.51 ± 9.37	15.04 ± 5.90	11.13 ± 9.98	16.24 ± 10.32
No Stress	3.07 ± 8.54	4.22 ± 9.83	2.93 ± 6.87	5.20 ± 5.50	0.81 ± 11.27

**Table 6.4:** % Rise in Diastolic Blood Pressure post stressor in High/Low WHR Males/Females (Means ± SD)

Analysis of the percentage rise in systolic and diastolic blood pressure revealed a main effect of condition for both systolic ( $F(1, 61) = 80.056$ ;  $p < 0.01$ ) and diastolic blood pressure ( $F(1, 61) = 30.108$ ;  $p < 0.01$ ). A greater percentage rise was observed in the stress condition for both systolic and diastolic blood pressure. No other significant interactions or main effects for waist-hip ratio or gender were observed.

#### 6.4.2.6 Change from Baseline Blood Pressure (Anticipation & Recovery)

As in the analysis of patterns of cortisol activity, patterns of blood pressure at various time points were assessed in terms of anticipation and recovery.

##### i. Anticipation

Anticipation was calculated as the change from baseline to the blood pressure reading obtained at time 2 (post preparation and pre-stress/no stress task). Anticipation was the

dependent variable in a 2x2x2 between subjects ANOVA with waist-hip ratio, gender and condition as between subjects' factors.

A main effect of condition on anticipation in both systolic and diastolic blood pressure was observed (systolic:  $F(1, 61) = 55.822$ ;  $p < 0.01$  and diastolic:  $F(1, 61) = 30.177$ ;  $p < 0.01$ ). Greater anticipation was observed in the stress condition compared to the no stress condition for both systolic ( $17.03 \pm 1.32$  and  $3.01 \pm 1.34$  mmHg respectively) and diastolic blood pressure ( $9.94 \pm 1.14$  and  $1.03 \pm 1.15$  mmHg respectively). No other significant interactions or main effects for waist-hip ratio or gender were observed.

## ii. Recovery

Recovery was calculated as change from baseline to the final blood pressure reading obtained (time 6). Recovery was the dependent variable in a 2x2x2 between subjects ANOVA with waist-hip ratio, gender and condition as between subjects' factors.

A main effect of condition was observed for recovery (change from baseline from time point 1 to time point 6) of systolic blood pressure ( $F(1, 61) = 13.415$ ;  $p < 0.01$ ). Less recovery was observed in the stress condition compared to the no stress condition ( $8.28 \pm 1.35$  and  $1.24 \pm 1.37$  mmHg respectively). Analysis of diastolic blood pressure demonstrated only a trend for a difference ( $p = 0.055$ ) with less recovery observed in the stress condition compared to the no stress condition ( $6.94 \pm 1.18$  and  $3.62 \pm 1.22$  mmHg respectively).

### 6.4.2.7 STAI and SSES Scores in High/Low WHR Males/Females by Stress/No Stress Condition

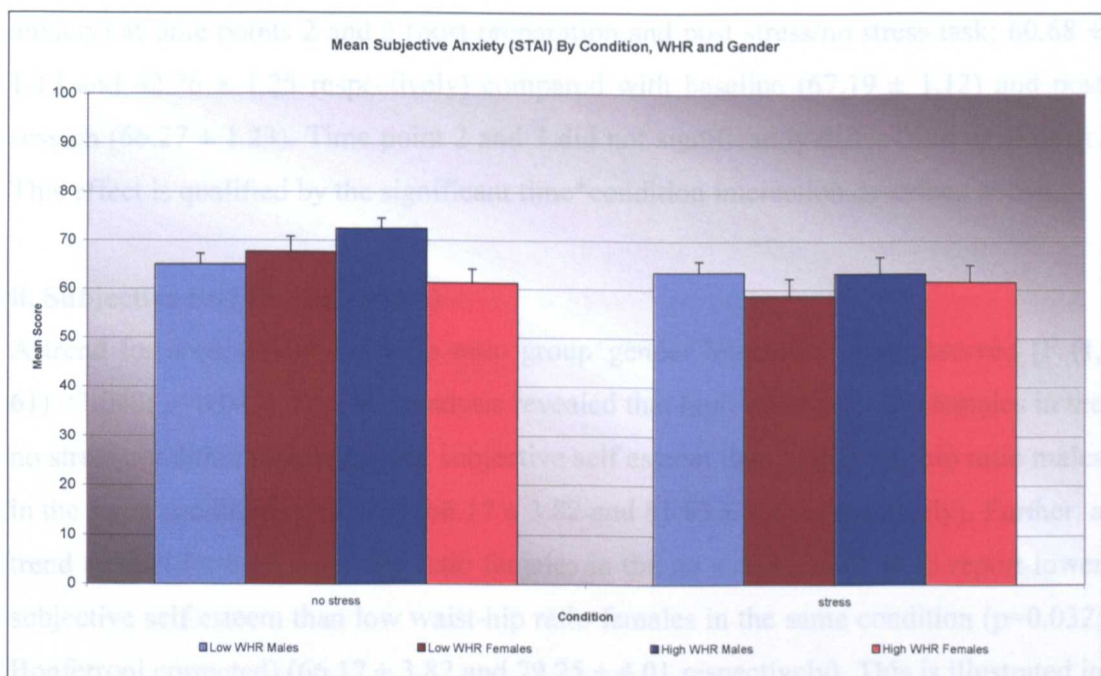
Subjective anxiety and self-esteem (as measured by the State Trait Anxiety Inventory and the State Self Esteem Scale) were assessed across the test session at baseline, post preparation, post task and post session to monitor change in anxiety and self esteem in each condition. All 70 observations were included in the analysis.

#### i. Anxiety (STAI)

Analysis of covariance revealed age was a significant covariate for subjective anxiety score over the duration of the test session ( $F(1, 61) = 8.581$ ;  $p < 0.01$ ). A linear regression was conducted to assess the strength of the relationship between and the dependent

variable. Age was found to provide significant adjustment to STAI score at three of the four assessment time points. Specifically, the beta ( $\beta$ ) value of 0.51 at time 1 ( $t(68) = 3.356$ ;  $p < 0.01$ ), 0.59 at time 2 ( $t(68) = 3.375$ ;  $p < 0.01$ ) and 0.37 at time 3 ( $t(68) = 2.047$ ;  $p < 0.01$ ) significantly differed from zero. This indicated that STAI was associated with age, such that an increase in score for the STAI (less anxiety) was associated with increased age in this sample.

A trend for a condition\*waist-hip ratio group\*gender interaction was observed ( $F(1, 61) = 3.223$ ;  $p = 0.078$ ). However, Bonferroni corrected t-tests failed to reach significance. Based on the means, there was a tendency for low waist-hip ratio males in the no stress condition to report greater subjective anxiety (indicated by a lower score) than high waist-hip ratio males in the same condition ( $72.50 \pm 2.06$  and  $65.08 \pm 2.25$  respectively). Further, high waist-hip ratio males in the no stress condition reported greater subjective anxiety than high waist-hip ratio males in the stress condition ( $72.50 \pm 2.06$  and  $63.44 \pm 3.35$  respectively). This is illustrated in Figure 6.10 below.



**Figure 6.10:** STAI Scores in High/Low WHR Males/Females by Stress/No Stress Condition (Means  $\pm$  SEM)

A significant time\*condition interaction was also observed ( $F(3, 183) = 18.662$ ;  $p < 0.01$ ). Post hoc analyses revealed that subjective anxiety at time points 2 ( $p = 0.001$ ) and 3

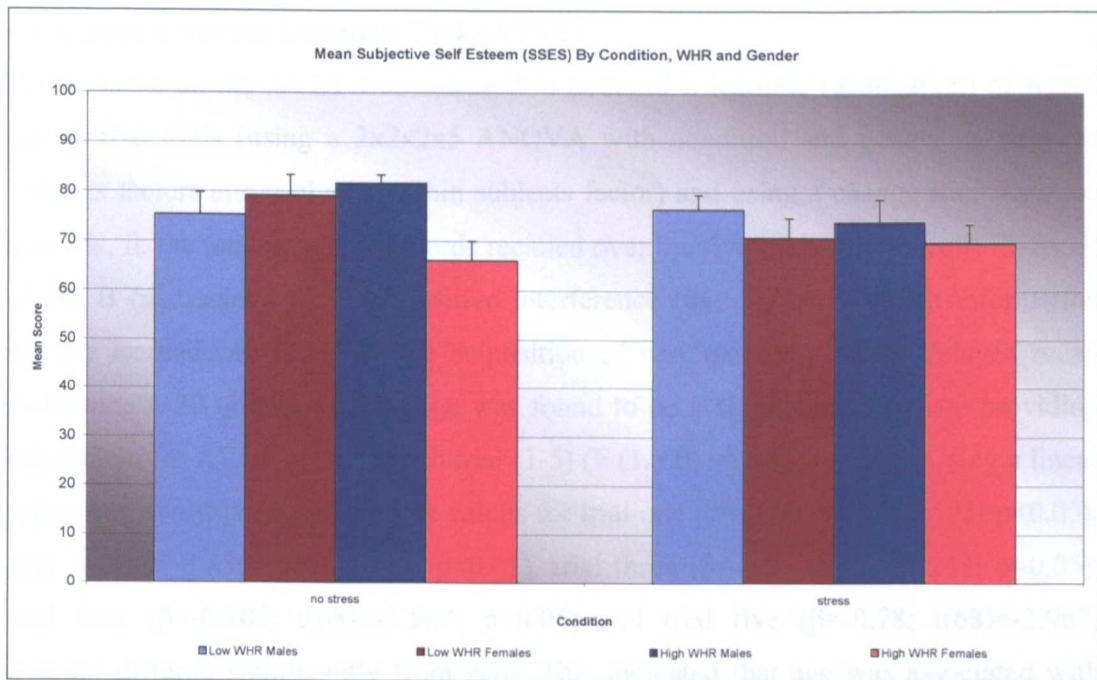
( $p=0.001$ ) differed significantly by condition. Subjective anxiety was consistently greater in the stress condition than in the no stress condition.

The condition\*waist-hip ratio group interaction ( $F(1, 61) = 0.045$ ;  $p=0.832$ ; NS), condition\*gender interaction ( $F(1, 61) = 0.000$ ;  $p=0.990$ ; NS) and waist-hip ratio\*gender interaction ( $F(1, 61) = 0.292$ ;  $p=0.591$ ; NS) all failed to reach significance. A main effect of condition was observed ( $F(1, 61) = 5.908$ ;  $p<0.05$ ). Greater anxiety was reported in the stress condition compared with the no stress condition ( $61.69 \pm 1.46$  and  $66.76 \pm 1.49$  respectively). A main effect of gender was also observed ( $F(1, 61) = 4.477$ ;  $p<0.05$ ). Females reported greater subjective anxiety compared with males ( $62.01 \pm 1.39$  and  $66.44 \pm 1.56$  respectively). No main effect of waist-hip ratio was observed ( $F(1, 61) = 0.025$ ;  $p=0.875$ ; NS).

A within subjects main effect of time was observed ( $F(3, 183) = 7.913$ ;  $p<0.01$ ) indicating that subjective anxiety altered across the test period. Pairwise comparisons revealed that subjective anxiety score was significantly lower (indicating greater anxiety) at time points 2 and 3 (post preparation and post stress/no stress task;  $60.68 \pm 1.15$  and  $62.76 \pm 1.25$  respectively) compared with baseline ( $67.19 \pm 1.12$ ) and post session ( $66.27 \pm 1.23$ ). Time point 2 and 3 did not significantly differ from each other. This effect is qualified by the significant time\*condition interaction described above.

## ii. Subjective Self Esteem (SSES)

A trend for a condition\*waist-hip ratio group\*gender interaction was observed ( $F(1, 61) = 3.040$ ;  $p=0.083$ ). Post hoc analysis revealed that high waist-hip ratio females in the no stress condition reported lower subjective self esteem than high waist-hip ratio males in the same condition ( $p=0.003$ ) ( $66.17 \pm 3.82$  and  $81.83 \pm 1.61$  respectively). Further, a trend existed for high waist-hip ratio females in the no stress condition to report lower subjective self esteem than low waist-hip ratio females in the same condition ( $p=0.032$ ; Bonferroni corrected) ( $66.17 \pm 3.82$  and  $79.25 \pm 4.01$  respectively). This is illustrated in Figure 6.11 below.



**Figure 6.11:** SSES Scores in High/Low WHR Males/Females by Stress/No Stress Condition (Means  $\pm$  SEM)

The condition\*waist-hip ratio group interaction ( $F(1, 61) = 0.006$ ;  $p = 0.941$ ; NS), condition\*gender interaction ( $F(1, 61) = 0.003$ ;  $p = 0.959$ ; NS) and waist-hip ratio\*gender interaction ( $F(1, 61) = 1.485$ ;  $p = 0.228$ ; NS) all failed to reach significance.

A main effect of gender was observed ( $F(1, 61) = 4.220$ ;  $p < 0.05$ ), lower subjective self-esteem was reported by females ( $71.32 \pm 1.88$ ) compared to males ( $77.13 \pm 2.11$ ). No main effect of condition ( $F(1, 61) = 1.176$ ;  $p = 0.282$ ; NS) or waist-hip ratio ( $F(1, 61) = 1.389$ ;  $p = 0.243$ ; NS) was observed. Age was not a significant covariate ( $F(1, 61) = 1.977$ ;  $p = 0.165$ ; NS).

#### 6.4.2.8 Cognitive Performance in High/Low WHR Males/Females by Stress/No Stress Condition

Performance on each task was assessed separately using a 2x2x2 ANOVA with condition, waist-hip ratio group and gender as between subjects factors. The score on each cognitive task was the dependent variable in each analysis. Age and predicted IQ were covariates. All 70 observations were included in each analysis. Performance on the auditory verbal learning task is considered first followed by the tasks employed from the Cambridge Automated Neuropsychological Test Battery (CANTAB). For a table of the associated F-Values (for CANTAB) refer to Appendix 20.

**(a) Auditory Verbal Learning Task (AVLT)**

Performance on the AVLT was assessed in terms of i. learning (acquisition) of words across five trials (using a 2x2x2x5 ANOVA with condition and gender as between subjects factors and trial as a within subjects factor) and using a change from baseline analysis, ii. the total number of words recalled over the five trials (total recall) iii. recall of list B (distracter list) iv. pro-active interference (the degree to which information already learned can influence the acquisition of new material) and v. delayed recall (following a 20 minute delay). Age was found to be a significant covariate providing adjustment for AVLT score at each trial (1-5) ( $F(1, 60) = 8.432; p < 0.01$ ). Using a linear regression analysis, the given beta values for trial one ( $\beta = -0.90; t(68) = -2.595; p < 0.05$ ), trial two ( $\beta = -0.85; t(68) = -2.217; p < 0.05$ ), trial three ( $\beta = -0.95; t(68) = -3.242; p < 0.05$ ), trial four ( $\beta = -0.104; t(68) = -3.567; p < 0.05$ ) and trial five ( $\beta = -0.78; t(68) = -2.967; p < 0.05$ ) differed significantly from zero. This indicated that age was associated with score on each of the trials, such that an increase in age was associated with a decrease in recall on each trial. IQ was not a significant covariate ( $F(1, 60) = 2.213; p = 0.142; NS$ ).

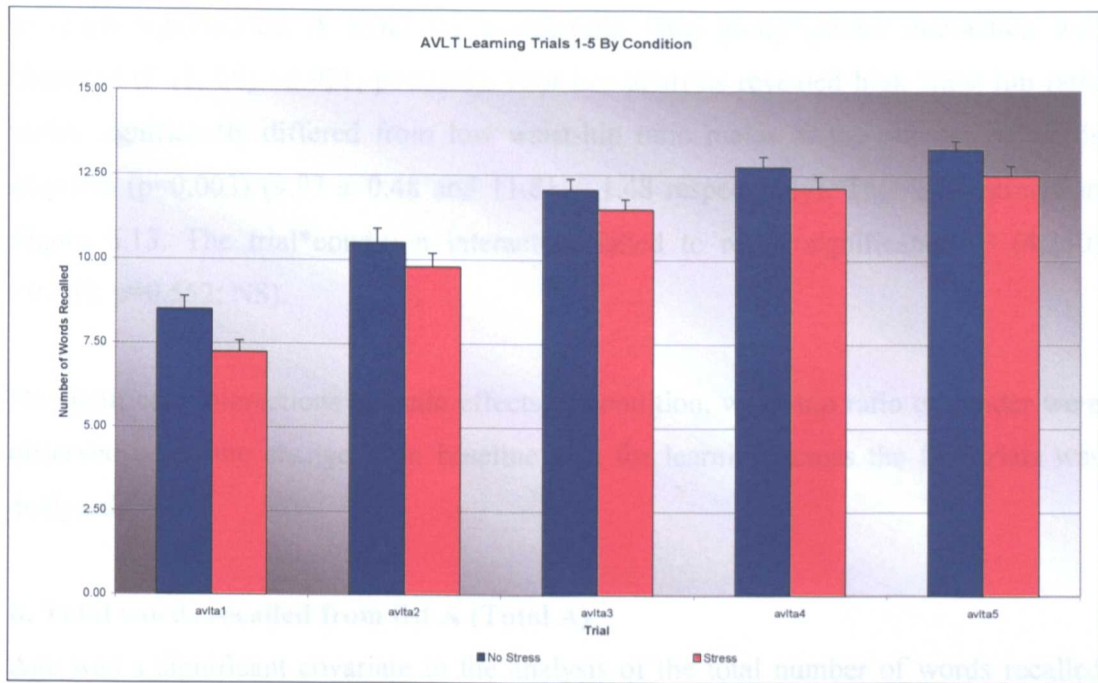
**i. Learning**

A main effect of trial on the number of words acquired across the five trials failed to reach significance ( $F(4, 240) = 2.004; p = 0.102; NS$ ). However, mean word recall across the five trials increases in from trial one to trial five, which demonstrates a clear learning curve. This is illustrated in Figure 6.12 below.

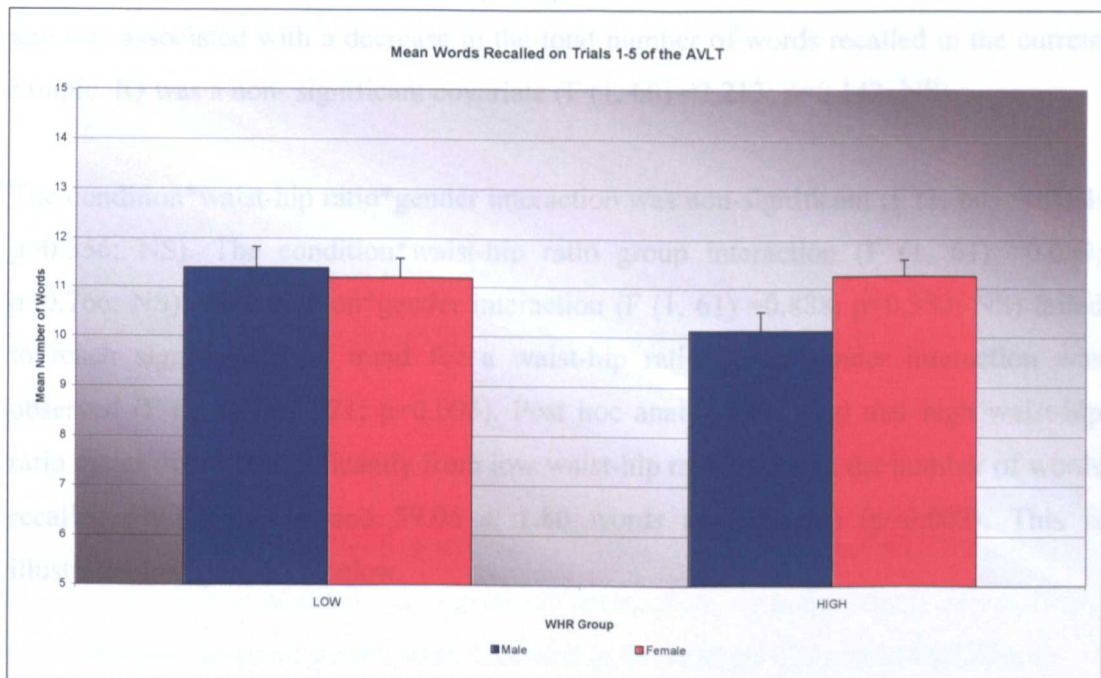
A trend for a main effect of condition was observed ( $F(1, 60) = 3.899; p = 0.053$ ). More words were acquired on average in the no stress condition compared with the stress condition ( $11.37 \pm 0.27$  and  $10.63 \pm 0.26$  respectively) (Figure 6.12).

No main effect of waist-hip ratio group ( $F(1, 60) = 2.410; p = 0.126; NS$ ) or gender ( $F(1, 60) = 1.348; p = 0.250; NS$ ) was observed.





**Figure 6.12:** Mean number of words recalled on Trials 1-5 of the AVLT by Stress/No Stress condition (Means  $\pm$  SEM)



**Figure 6.13:** Mean number of words recalled from Trials 1-5 of the AVLT in High/Low WHR Males/Females (Means  $\pm$  SEM)

The condition\*waist-hip ratio\*gender interaction was non-significant ( $F(1, 60) = 0.864$ ;  $p = 0.356$ ; NS). The condition\*waist-hip ratio group interaction ( $F(1, 61) = 0.006$ ;  $p = 0.941$ ; NS) and condition\*gender interaction ( $F(1, 61) = 0.003$ ;  $p = 0.959$ ; NS) failed

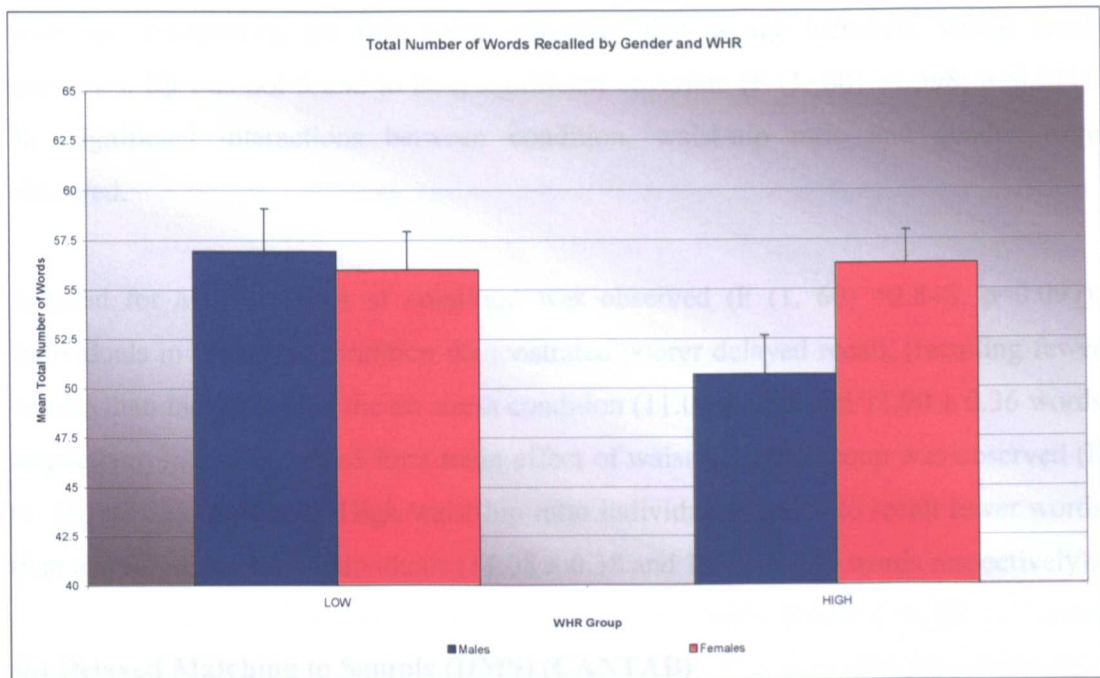
to reach significance. A trend for a waist-hip ratio group\*gender interaction was observed ( $F(1, 60) = 2.921$ ;  $p = 0.093$ ). Post hoc analysis revealed high waist-hip ratio males significantly differed from low waist-hip ratio males in the number of words acquired ( $p = 0.003$ ) ( $9.93 \pm 0.48$  and  $11.81 \pm 1.48$  respectively). This is illustrated in Figure 6.13. The trial\*condition interaction failed to reach significance ( $F(4, 240) = 0.760$ ;  $p = 0.552$ ; NS).

No significant interactions or main effects of condition, waist-hip ratio or gender were observed when the change from baseline data for learning across the five trials was analysed.

#### ii. Total words recalled from list A (Total A)

Age was a significant covariate in the analysis of the total number of words recalled across trials 1-5 ( $F(1, 60) = 8.432$ ;  $p < 0.01$ ). The beta value of  $-0.451$  for age differed significantly from zero ( $t(68) = -3.445$ ;  $p < 0.05$ ). This indicated that age was associated with verbal recall on the AVLT. Interpreting the beta value indicated that an increase in age was associated with a decrease in the total number of words recalled in the current sample. IQ was a non-significant covariate ( $F(1, 60) = 2.213$ ;  $p = 0.142$ ; NS).

The condition\*waist-hip ratio\*gender interaction was non-significant ( $F(1, 60) = 0.864$ ;  $p = 0.356$ ; NS). The condition\*waist-hip ratio group interaction ( $F(1, 61) = 0.089$ ;  $p = 0.766$ ; NS) and condition\*gender interaction ( $F(1, 61) = 0.888$ ;  $p = 0.350$ ; NS) failed to reach significance. A trend for a waist-hip ratio group\*gender interaction was observed ( $F(1, 60) = 2.921$ ;  $p = 0.093$ ). Post hoc analysis revealed that high waist-hip ratio males differed significantly from low waist-hip ratio males in the number of words recalled ( $49.64 \pm 2.41$  and  $59.06 \pm 1.80$  words respectively) ( $p = 0.003$ ). This is illustrated in Figure 6.14 below.



**Figure 6.14:** Total number of words recalled from List A in High/Low WHR Males/Females (Means  $\pm$  SEM)

A trend for a main effect of condition was observed ( $F(1, 60) = 3.899$ ;  $p = 0.053$ ). Individuals in the stress condition recalled fewer words in total than individuals in the no stress condition ( $53.14 \pm 1.31$  and  $56.84 \pm 1.33$  words respectively).

### iii. Recall of list B

No significant interactions or main effects of condition, waist-hip ratio group or gender were observed in the analysis of the number of words recalled from List B (distracter list).

### iv. Proactive Inhibition

Proactive Interference is the potential for information already learned to influence the acquisition of new material. No significant interactions or main effects of condition, waist-hip ratio group or gender were observed in the analysis of proactive inhibition.

### v. Delayed Recall of List A

The number of words recalled after a 20minute delay assessed delayed recall performance. Age was found to be a significant covariate ( $F(1, 60) = 12.013$ ;  $p < 0.01$ ). Using a linear regression analysis, the beta value of  $-0.137$  differed significantly from zero ( $t(68) = -3.964$ ;  $p < 0.05$ ). This indicated that delayed verbal recall was associated

with age. Interpreting the beta value suggested that as age increases, verbal recall decreases. IQ was not found to be a significant covariate ( $F(1, 60) = 1.598$ ;  $p = 0.211$ ). No significant interactions between condition, waist-hip ratio and gender were observed.

A trend for a main effect of condition was observed ( $F(1, 60) = 2.846$ ;  $p = 0.097$ ). Individuals in the stress condition demonstrated poorer delayed recall, (recalling fewer words) than individuals in the no stress condition ( $11.04 \pm 0.35$  and  $11.90 \pm 0.36$  words respectively). A minor trend for a main effect of waist-hip ratio group was observed ( $F(1, 60) = 2.235$ ;  $p = 0.140$ ). High waist-hip ratio individuals tended to recall fewer words than low waist-hip ratio individuals ( $11.08 \pm 0.38$  and  $11.90 \pm 0.34$  words respectively).

### **(b) Delayed Matching to Sample (DMS) (CANTAB)**

Performance on the delayed matching to sample task was assessed using three scores; i. the overall percentage correct regardless of the length of delay between viewing the pattern and making a choice from four variations of the target pattern, ii. the percentage correct when the pattern and variant choices appear at the same time and iii. the probability of failure following an error on a preceding trial or a correct response on a preceding trial.

#### **i. Percentage correct all delays**

IQ was a significant covariate ( $F(1, 60) = 4.471$ ;  $p < 0.05$ ). Using a linear regression analysis, the beta value differed significantly from zero ( $t(68) = 2.656$ ;  $p < 0.05$ ). This indicated that IQ was associated with performance on the delayed matching to sample task. Interpretation of the beta value suggested that an increase in IQ was associated with better performance on the task. Age was not found to be a significant covariate ( $F(1, 60) = 0.620$ ;  $p = 0.434$ ; NS). No significant interactions between condition, waist-hip ratio and gender were observed.

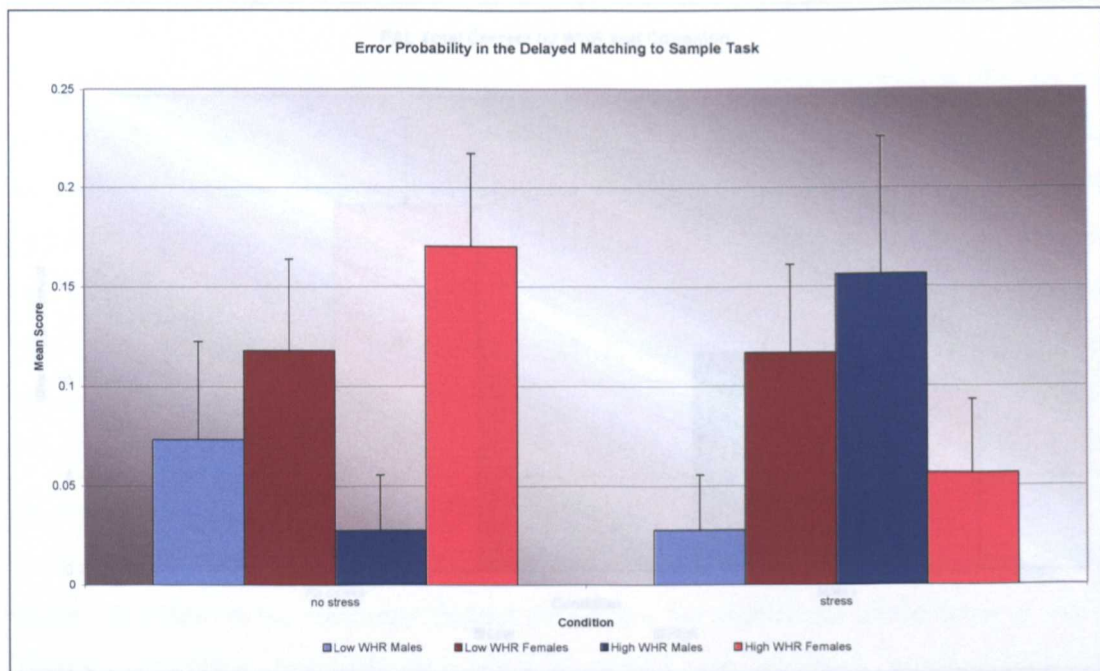
A trend for a main effect of gender was observed ( $F(1, 60) = 3.353$ ;  $p = 0.072$ ). Males demonstrated better performance in terms of correct responses, compared with females (87.32% correct  $\pm 1.75$  and 82.89% correct  $\pm 1.55$  respectively). No main effect of waist-hip ratio ( $F(1, 60) = 0.708$ ;  $p = 0.403$ ; NS) or condition ( $F(1, 60) = 1.624$ ;  $p = 0.207$ ; NS) were observed.

### ii. Percentage correct Simultaneous

In the analysis of percentage correct responses when target and variant choices were presented simultaneously, no significant interactions or main effects of condition, waist-hip ratio group or gender were observed. Age and IQ were not found to be significant covariates.

### iii. Error probability

Error probability was a score to indicate the likelihood of making an error based on previous responses. A significant condition\*waist-hip ratio group\*gender interaction was observed ( $F(1, 60) = 4.581; p < 0.05$ ). However, Bonferroni corrected t-tests failed to reach significance. Based on means, high waist-hip ratio females in the no stress condition demonstrated a greater error probability than high waist-hip ratio females in the stress condition ( $0.17 \pm 0.05$  and  $0.06 \pm 0.04$  respectively). In contrast, high waist-hip ratio males in the stress condition demonstrated greater error probability than high waist-hip ratio males in the no stress condition ( $0.16 \pm 0.07$  and  $0.03 \pm 0.03$  respectively). This is illustrated in Figure 6.15 below.



**Figure 6.15:** DMS Error Probability in High/Low WHR Males/Females by Stress/No Stress Condition (Means  $\pm$  SEM)

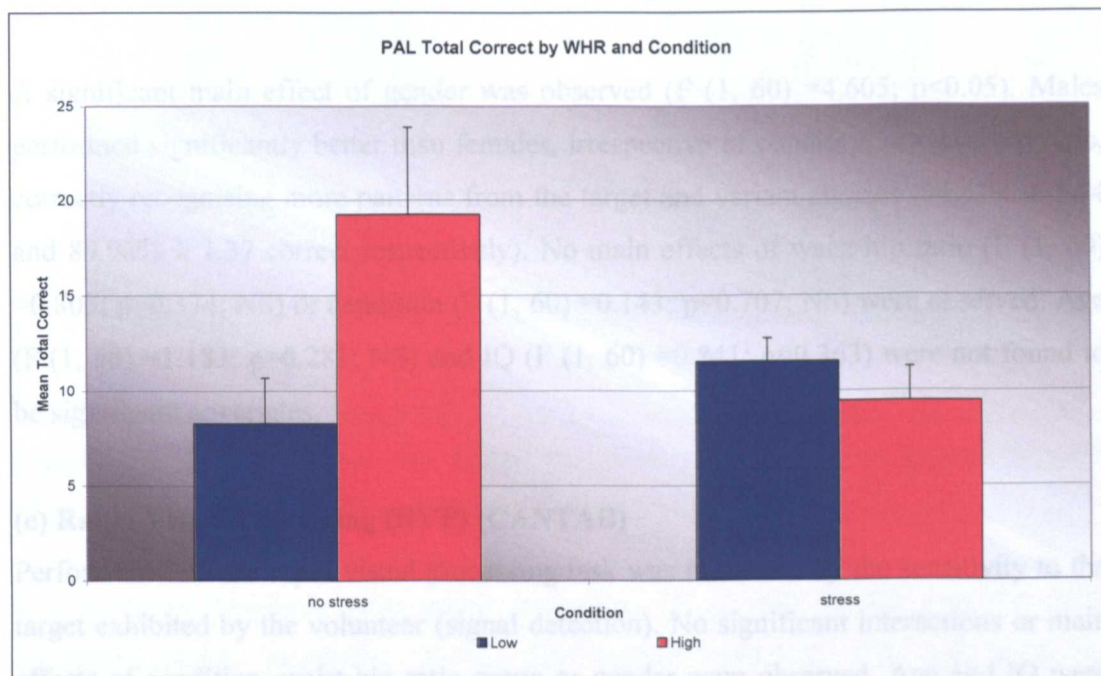
Figure 6.15 suggests that low waist-hip ratio females do not change across condition but high waist-hip ratio females do, making fewer errors under stress (lower error probability). The opposite is true for high waist-hip ratio males, while low waist-hip ratio males show little change.

The condition\*waist-hip ratio group interaction ( $F(1, 60) = 0.036$ ;  $p = 0.850$ ; NS), condition\*gender interaction ( $F(1, 60) = 2.091$ ;  $p = 0.153$ ; NS) and waist-hip ratio\*gender interaction ( $F(1, 60) = 0.373$ ;  $p = 0.543$ ; NS) failed to reach significance.

No significant main effect of condition ( $F(1, 60) = 0.063$ ;  $p = 0.803$ ; NS), gender ( $F(1, 60) = 0.856$ ;  $p = 0.359$ ; NS) or waist-hip ratio group ( $F(1, 60) = 0.117$ ;  $p = 0.734$ ; NS) were observed. Age ( $F(1, 60) = 0.285$ ;  $p = 0.595$ ; NS) and IQ ( $F(1, 60) = 0.468$ ;  $p = 0.497$ ; NS) were not found to be significant covariates.

### (c) Paired Associates Learning (PAL) (CANTAB)

Performance on the paired associates learning task was determined by the total number of correct responses (number of completed trials).



**Figure 6.16:** Total number of correct trials on the paired associates learning task (PAL) in High/Low WHR by Stress/No Stress condition (Means  $\pm$  SEM)

The condition\*waist-hip ratio\*gender interaction was non-significant ( $F(1, 60) = 0.708$ ;  $p = 0.403$ ; NS). A significant condition\*waist-hip ratio group interaction was observed ( $F(1, 60) = 6.194$ ;  $p < 0.05$ ). Post hoc analyses revealed that poorer performance post stress was found in high waist-hip individuals compared with high waist-hip ratio individuals in the no stress condition ( $9.44 \pm 1.78$  and  $19.27 \pm 4.62$  words respectively). This is illustrated in Figure 6.16.

The condition\*gender interaction ( $F(1, 60) = 0.894$ ;  $p = 0.348$ ; NS) and waist-hip ratio\*gender interaction ( $F(1, 60) = 0.108$ ;  $p = 0.744$ ; NS) failed to reach significance. No significant main effect of condition ( $F(1, 60) = 2.159$ ;  $p = 0.147$ ; NS), gender ( $F(1, 60) = 0.264$ ;  $p = 0.609$ ; NS) or waist-hip ratio group ( $F(1, 60) = 2.018$ ;  $p = 0.161$ ; NS) were observed. Age ( $F(1, 60) = 2.310$ ;  $p = 0.134$ ; NS) and IQ ( $F(1, 60) = 0.045$ ;  $p = 0.834$ ; NS) were not found to be significant covariates.

#### **(d) Pattern Recognition Memory (PRM) (CANTAB)**

Performance on the Pattern Recognition Memory task was determined by the percentage of correctly recognised patterns from a previously learned list. No significant interactions between condition, waist-hip ratio and gender were observed.

A significant main effect of gender was observed ( $F(1, 60) = 4.605$ ;  $p < 0.05$ ). Males performed significantly better than females, irrespective of condition or waist-hip ratio, correctly recognising more patterns from the target and variant choices ( $94.53\% \pm 1.54$  and  $89.96\% \pm 1.37$  correct respectively). No main effects of waist-hip ratio ( $F(1, 60) = 0.803$ ;  $p = 0.374$ ; NS) or condition ( $F(1, 60) = 0.143$ ;  $p = 0.707$ ; NS) were observed. Age ( $F(1, 60) = 1.183$ ;  $p = 0.281$ ; NS) and IQ ( $F(1, 60) = 0.841$ ;  $p = 0.363$ ) were not found to be significant covariates.

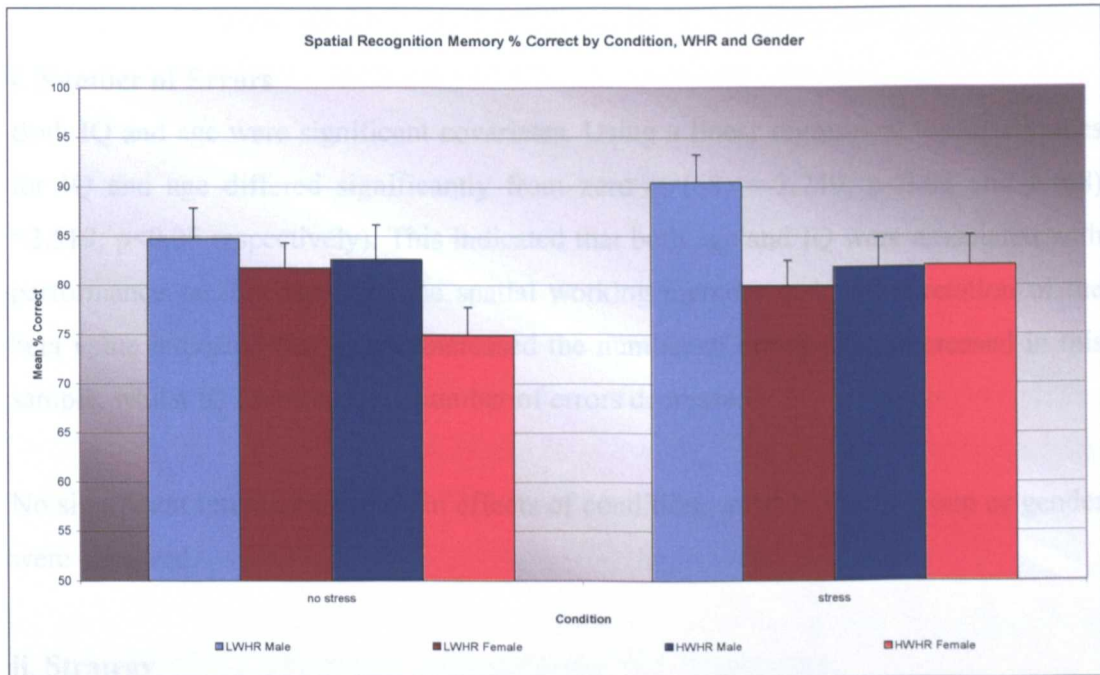
#### **(e) Rapid Visual Processing (RVP) (CANTAB)**

Performance on the rapid visual processing task was measured by the sensitivity to the target exhibited by the volunteer (signal detection). No significant interactions or main effects of condition, waist-hip ratio group or gender were observed. Age and IQ were not found to be significant covariates.

**(f) Spatial Recognition Memory (SRM) (CANTAB)**

Performance on the spatial recognition task was assessed in terms of the percentage of correctly recognised locations across all trials.

A trend for a condition\*waist-hip ratio group\*gender interaction was observed ( $F(1, 60) = 3.556$ ;  $p = 0.064$ ). However, Bonferroni corrected t-tests failed to reach significance. Based on means, high waist-hip ratio males in the stress condition correctly recognised fewer locations (expressed as percentage correct) than low waist-hip ratio males in the same condition who performed better under stress ( $82.50\% \pm 2.83$  and  $90.63\% \pm 1.75$  respectively). The low waist-hip ratio group appeared to perform better than other groups both under stress and under no stress. This is illustrated in Figure 6.17 below.



**Figure 6.17:** % Correct on the spatial recognition task (SRM) in High/Low WHR Males/Females by Stress/No Stress Condition (Means  $\pm$  SEM)

The condition\*waist-hip ratio group interaction ( $F(1, 60) = 0.083$ ;  $p = 0.774$ ; NS), condition\*gender interaction ( $F(1, 60) = 0.001$ ;  $p = 0.978$ ; NS) and waist-hip ratio\*gender interaction ( $F(1, 60) = 0.494$ ;  $p = 0.485$ ; NS) failed to reach significance.

A main effect of gender was observed ( $F(1, 60) = 5.761$ ;  $p < 0.05$ ). Males performed significantly better than females, remembering a higher percentage of previously



learned locations ( $85.00 \pm 1.58$  and  $79.65 \pm 1.40$  respectively). A trend for a main effect of waist-hip ratio group was observed ( $F(1, 60) = 3.376$ ;  $p = 0.071$ ). Low waist-hip ratio individuals demonstrated better performance than high waist-hip ratio individuals ( $84.20 \pm 1.38$  and  $80.34 \pm 1.54$  respectively). No main effect of condition was observed ( $F(1, 60) = 1.563$ ;  $p = 0.216$ ; NS). Age ( $F(1, 60) = 1.294$ ;  $p = 0.260$ ; NS) and IQ ( $F(1, 60) = 2.535$ ;  $p = 0.117$ ; NS) were not found to be significant covariates.

#### **(g) Spatial Working Memory (SWM) (CANTAB)**

Performance on the spatial working memory task was assessed using two measures; i. the number of errors made across trials in selecting an incorrect target location or re-selecting a previously used target location and ii. an estimation of the probability that the volunteer implemented some form of strategy to aid successful location of the target blue tokens.

##### **i. Number of Errors**

Both IQ and age were significant covariates. Using a linear regression, the beta values for IQ and age differed significantly from zero ( $t(68) = -2.749$ ;  $p < 0.05$  and  $t(68) = 2.519$ ;  $p < 0.05$  respectively). This indicated that both age and IQ were associated with performance on this aspect of the spatial working memory task. Interpretation of the beta value indicated that as age increased the number of errors made increased in this sample, whilst IQ increased, the number of errors decreased.

No significant interactions or main effects of condition, waist-hip ratio group or gender were observed.

##### **ii. Strategy**

Analysis of the probability that a strategy was implemented to aid successful location of the blue tokens revealed no significant interactions or main effects of condition, waist-hip ratio group or gender were observed. Age and IQ were not found to be significant covariates.

#### **(h) Stockings of Cambridge (SOC) (CANTAB)**

The Stockings of Cambridge task similar to the Tower of London task. Performance is assessed in terms of the minimum number of moves taken to solve a problem. It was

also of interest in this task to explore; i. the mean initial thinking time for a 5-move problem and ii. the mean subsequent thinking time after the first move in a 5-move problem.

**i. Minimum number of moves taken to solve a problem**

No significant interactions of condition, waist-hip ratio group or gender were observed. Age and IQ were not found to be significant covariates. A significant main effect of gender was observed ( $F(1, 60) = 15.421; p < 0.01$ ). Males required significantly more moves to solve a 5-move problem than females ( $10.10 \pm 0.33$  and  $8.02 \pm 0.29$  respectively). No main effect of waist-hip ratio ( $F(1, 60) = 1.079; p = 0.303; NS$ ) or condition ( $F(1, 60) = 1.278; p = 0.263; NS$ ) were observed.

**ii. Mean initial thinking time (5 moves)**

No significant interactions or main effects of condition, waist-hip ratio group or gender were observed. Age and IQ were not found to be significant covariates.

**iii. Mean subsequent thinking time (following the first move in a 5-move problem)**

A significant condition\*waist-hip ratio group\*gender interaction was observed ( $F(1, 60) = 3.993; p < 0.05$ ). However, Bonferroni corrected t-tests failed to reach significance. Based on means, high waist-hip ratio females in the no stress condition had longer thinking time between the first and second moves than low waist-hip ratio females in the same condition ( $2025.60 \pm 470.18$  and  $891.02 \pm 202.70$  respectively). This is a large mean difference but is more variable in the high waist-hip ratio females. No other differences in terms of condition or waist-hip ratio were noted.

**6.4.2.9 Cognitive Performance in Responders/Non-Responders**

The assessment of cognitive performance according to stress/no stress condition does not reflect cortisol activity. It cannot be assumed that each individual in the stress condition demonstrated a cortisol response to stress and no response occurred in the no stress condition. Frequencies of responders by waist-hip ratio and gender are shown in Table 6.5. Hence, cognitive performance was subsequently analysed using cortisol response group (responders versus non-responders) irrespective of stress/no stress condition. A median split was performed on the data for percentage rise in cortisol from baseline to the mean of cortisol at time 3 and 4, to split the sample into responders and

non-responders. The median cortisol response was an increase in cortisol of 13%. Therefore, any individuals exhibiting a response greater than or equal to 13% was classed as a responder and those whose response was less than 13%, a non-responder. Because of low cell occupancy for some waist-hip ratio – gender combinations, it was not possible to include stress/no stress condition in the analysis of cognitive performance in responders and non-responders.

	Stress		No Stress	
	Responder	Non Responder	Responder	Non Responder
HWHR Males	5	0	4	2
HWHR Females	4	1	0	9
LWHR Males	7	0	0	9
LWHR Females	7	3	4	6

**Table 6.5:** Frequency Table of Cortisol Responders & Non-Responders to stress induction

Table 6.5 indicates that the majority of individuals exposed to stress were cortisol responders, further, the majority of individuals in the no stress condition did not show a cortisol response with the exception of high waist-hip ratio males and low waist-hip ratio females who demonstrated more of a response to the no stress condition compared to the other subgroups.

The response to stress could also be identified by cortisol response combined with systolic blood pressure response, rather than cortisol response alone. The same median split procedure for cortisol was implemented, in conjunction with a median split for systolic blood pressure. A volunteer was classed as a ‘stress responder’ if a greater than median response was exhibited for both cortisol and blood pressure, (the median blood pressure response was 7%). If both responses did not meet this criteria, then the volunteer was classed as a non-responder. Cognitive performance was also assessed accordingly based on this categorisation. A frequency table for responders vs. non-responders is also shown in Table 6.6). A table for the associated F-values (for CANTAB) for both the cortisol response and combined cortisol and blood pressure response groups is shown in Appendix 21.

	Stress		No Stress	
	Responder	Non Responder	Responder	Non Responder
HWRH Males	5	0	0	6
HWRH Females	4	1	0	9
LWHR Males	6	1	0	9
LWHR Females	7	3	1	9

**Table 6.6:** Frequency Table of Stress Responders (Cortisol & Blood Pressure) & Non-Responders to stress induction

Table 6.6, as in Table 6.5, indicates that the majority of individuals exposed to stress were cortisol responders, further, the majority of individuals in the no stress condition did not show a cortisol response. However, the lack of response in the no stress condition was more consistent than when cortisol responses were considered with a high frequency of non-responders as would be expected. Due to missing data, 61 out of 70 observations were included in the analyses.

### (a) AVLT

#### i. Learning

##### -Cortisol Response Group

There were no significant interactions for cortisol response group\*waist-hip ratio group\*gender ( $F(1, 51) = 0.294$ ;  $p = 0.590$ ; NS), cortisol response group \*gender ( $F(1, 51) = 0.245$ ;  $p = 0.623$ ; NS) and cortisol response group\*waist-hip ratio ( $F(1, 51) = 0.284$ ;  $p = 0.596$ ; NS).

A trend for a main effect of cortisol response group was observed ( $F(1, 51) = 3.482$ ;  $p = 0.068$ ). Individuals who exhibited a cortisol response to the stress/no stress task showed poorer verbal recall than individuals who did not show a cortisol response over the five trials ( $10.66 \pm 0.32$  and  $11.06 \pm 0.38$  respectively).

##### -Stress Response Group (Cortisol and BP)

There were no significant interactions for stress response group\*waist-hip ratio group\*gender ( $F(1, 51) = 0.147$ ;  $p = 0.703$ ; NS), cortisol response group \*gender ( $F(1, 51) = 0.580$ ;  $p = 0.450$ ; NS) and cortisol response group\*waist-hip ratio ( $F(1, 51) = 0.857$ ;  $p = 0.359$ ; NS).

A trend for a main effect of stress response group was observed ( $F(1, 51) = 3.322$ ;  $p = 0.074$ ). As in cortisol responders, individuals who exhibited a stress response demonstrated poorer verbal recall (mean number of words recalled across trials 1-5) compared to stress non-responders ( $10.54 \pm 0.36$  and  $11.37 \pm 0.28$  respectively).

No significant interactions or main effects of response group (stress or cortisol), waist-hip ratio or gender were observed when analysing the data in terms of change from baseline.

## **ii. Total Recall of List A**

### **-Cortisol Response Group**

The cortisol response group\*waist-hip ratio group\*gender three way interaction ( $F(1, 51) = 0.294$ ;  $p = 0.590$ ; NS), cortisol response group \*gender interaction ( $F(1, 51) = 0.245$ ;  $p = 0.623$ ; NS) and cortisol response group\*waist-hip ratio interaction ( $F(1, 51) = 0.284$ ;  $p = 0.596$ ; NS) failed to reach significance.

A trend for a main effect of cortisol response group was observed ( $F(1, 51) = 3.482$ ;  $p = 0.068$ ). Those individuals who exhibited a cortisol response to the stress/no stress task demonstrated poorer verbal recall (total number of words recalled across trials 1-5) than individuals who did not show a cortisol response ( $53.32 \pm 1.61$  and  $58.02 \pm 1.91$  respectively).

### **-Stress Response Group (Cortisol and BP)**

The stress response group\*waist-hip ratio group\*gender three way interaction ( $F(1, 51) = 0.147$ ;  $p = 0.703$ ; NS), cortisol response group \*gender interaction ( $F(1, 51) = 0.580$ ;  $p = 0.450$ ; NS) and cortisol response group\*waist-hip ratio interaction ( $F(1, 51) = 0.857$ ;  $p = 0.359$ ; NS) failed to reach significance.

A trend for a main effect of stress response group was observed ( $F(1, 51) = 3.322$ ;  $p = 0.074$ ). Individuals who exhibited a stress response to the stress/no stress task demonstrated poorer verbal recall than individuals who did not show a cortisol response ( $52.72 \pm 1.78$  and  $56.82 \pm 1.38$  respectively).

**iii. Recall of List B****-Cortisol Response Group**

A significant main effect of cortisol response group was observed ( $F(1, 51) = 4.281$ ;  $p < 0.05$ ). Those individuals who exhibited a cortisol response to the stress/no stress task demonstrated poorer verbal recall of List B than individuals who did not show a cortisol response over the five trials ( $5.34 \pm 0.46$  and  $6.85 \pm 0.55$  respectively).

**-Stress Response Group (Cortisol and BP)**

No significant interactions of stress response group, waist-hip ratio and gender were observed. No main effect of stress response group was observed.

**iv. Proactive Inhibition**

No significant interactions or main effects of response group (stress or cortisol), waist-hip ratio or gender were observed.

**iii. Delayed Recall**

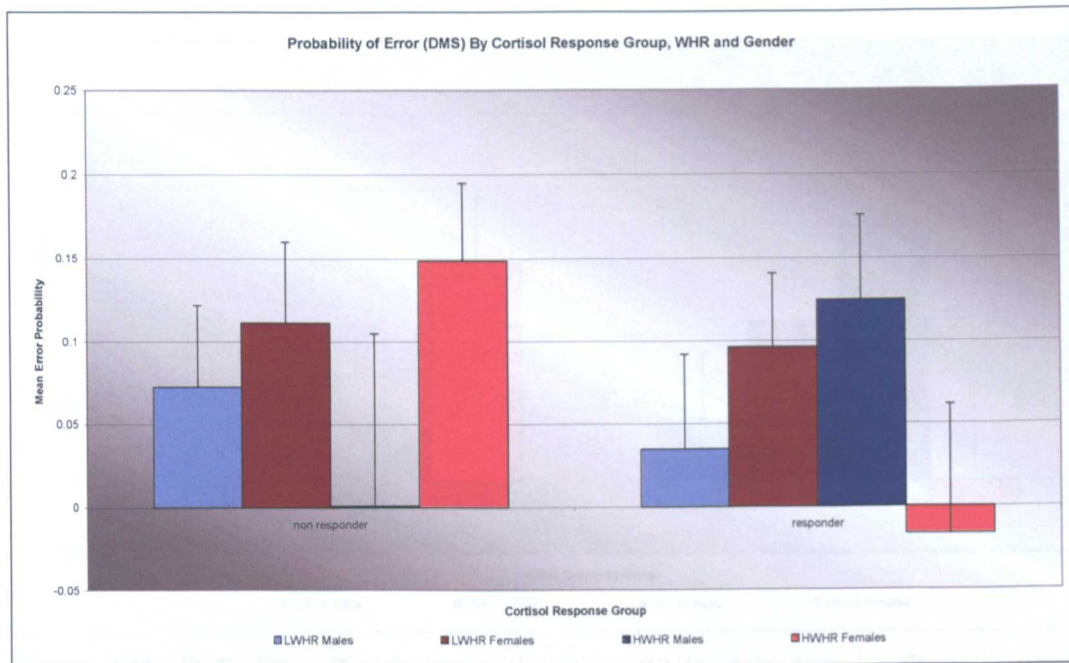
No significant interactions or main effects of response group (stress or cortisol), waist-hip ratio or gender were observed.

**(b) Delayed Matching to Sample (DMS)**

No significant interactions or main effects of response group (stress or cortisol), waist-hip ratio or gender were observed for the percentage of correct responses for all delays and the percentage correct when target and variant choices were presented simultaneously.

**- Cortisol Response Group**

A trend for a response group\*waist-hip ratio group\*gender interaction was observed ( $F(1, 51) = 3.098$ ;  $p = 0.084$ ) for error probability. Post hoc analysis revealed a significant difference between high waist-hip ratio female cortisol responders and non-responders ( $p = 0.008$ ). High waist-hip ratio female responders demonstrated a greater probability of making an error on the task than high waist-hip ratio female non-responders ( $0.15 \pm 0.14$  and  $0.00 \pm 0.00$  respectively). The opposite was true for high waist-hip ratio males, ( $0.12 \pm 0.06$  and  $0.00 \pm 0.00$  respectively) however this failed to reach significance ( $p = 0.437$ ). This is illustrated in Figure 6.18 below.

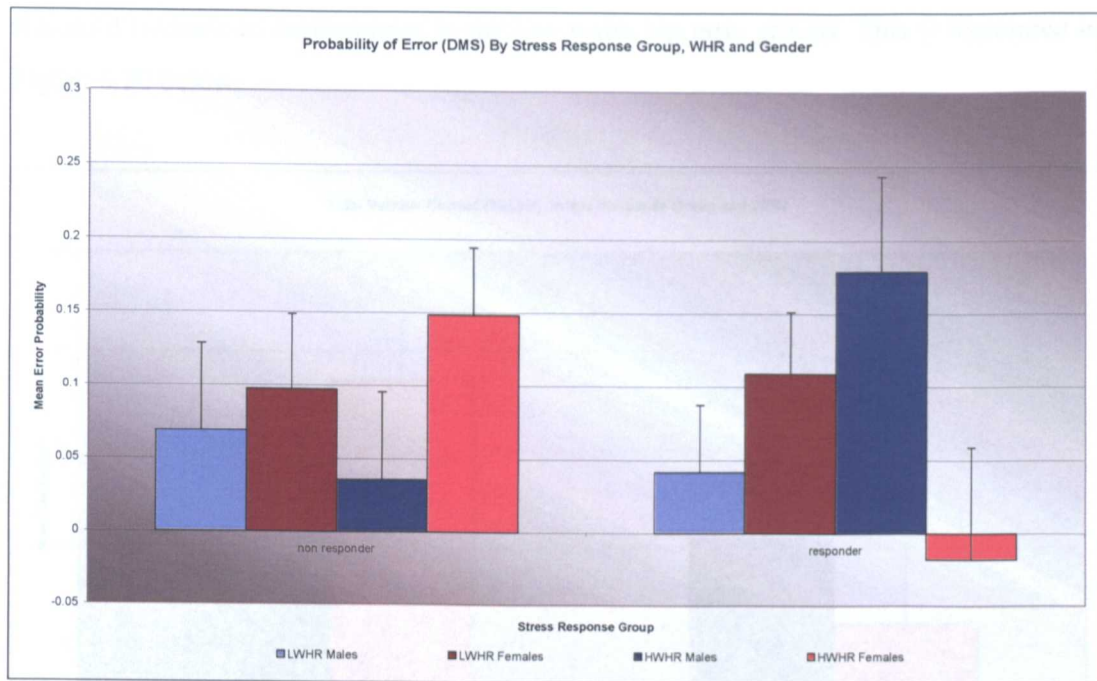


**Figure 6.18:** DMS Error Probability in High/Low WHR Males/Females Cortisol responders (Means  $\pm$  SEM)

The cortisol response group \*gender interaction ( $F(1, 51) = 2.343$ ;  $p = 0.132$ ; NS) and cortisol response group \*waist-hip ratio interaction ( $F(1, 51) = 0.004$ ;  $p = 0.948$ ; NS) failed to reach significance. No main effect of cortisol response group was observed ( $F(1, 51) = 0.279$ ;  $p = 0.600$ ).

#### - Stress Response Group (Cortisol and BP)

A significant stress response group \*waist-hip ratio group \*gender interaction was observed for the error probability ( $F(1, 51) = 4.870$ ;  $p < 0.05$ ). However, Bonferroni corrected t-tests failed to reach significance. Based on the means, high waist-hip ratio female stress responders demonstrated lower probability of error than high waist-hip ratio female non-responders ( $0.00 \pm 0.00$  and  $0.15 \pm 0.04$  respectively). Further, high waist-hip ratio female stress responders demonstrated a lower probability of error than low waist-hip ratio female responders ( $0.00 \pm 0.00$  and  $0.10 \pm 0.05$  respectively). Again, the opposite was true for high waist-hip ratio males, ( $0.11 \pm 0.07$  and  $0.06 \pm 0.06$  respectively) however this failed to reach significance ( $p = 0.680$ ). This is illustrated in Figure 6.18 below.



**Figure 6.19:** DMS Error Probability in High/Low WHR Males/Females Stress responders (combined cortisol & BP) (Means ± SEM)

Further, a trend for a stress response group\*gender interaction was observed ( $F(1, 51) = 2.987$ ;  $p = 0.090$ ). Post hoc analyses revealed a trend for female non-responders to demonstrate a greater probability of error than male non-responders ( $p = 0.091$ ) ( $0.13 \pm 0.03$  and  $0.05 \pm 0.03$  respectively). However, it is evident from Figure 6.19 that this is driven by the greater error probability in female high waist-hip ratio non-responders and in male high waist-hip ratio responders. The stress response group\*waist-hip ratio interaction ( $F(1, 51) = 0.002$ ;  $p = 0.963$ ; NS) failed to reach significance. No main effect of stress response group was observed ( $F(1, 51) = 0.063$ ;  $p = 0.803$ ).

### (c) Paired Associates Learning (PAL)

#### - Cortisol Response Group

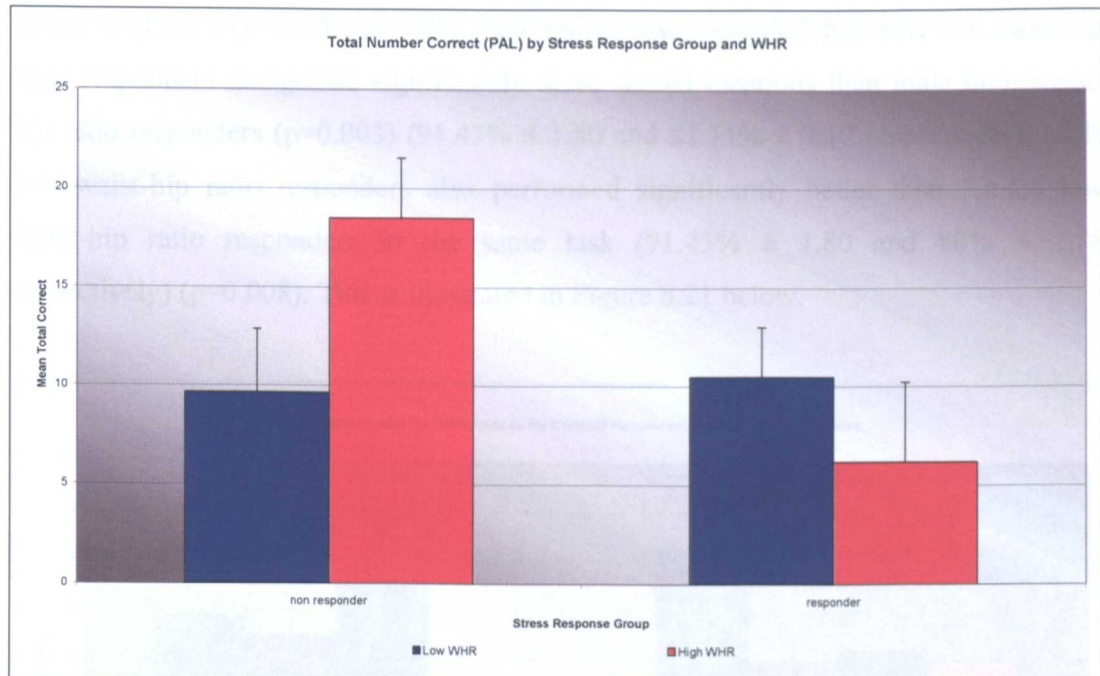
No significant interactions of cortisol response group, waist-hip ratio and gender were observed. No main effect of cortisol response group was observed.

#### - Stress Response Group (Cortisol and BP)

The stress response group\*waist-hip ratio group interaction was almost significant ( $F(1, 51) = 3.970$ ;  $p = 0.052$ ). Post hoc analyses revealed that high waist-hip ratio stress responders demonstrated significantly poorer performance than high waist-hip ratio non-responders ( $7.33 \pm 1.65$  and  $18.31 \pm 4.43$  words respectively) ( $p = 0.036$ ) but there



was no difference in performance in the low waist-hip ratio groups. This is illustrated in Figure 6.20 below.



**Figure 6.20:** Total number of correct trials on the paired associates learning task (PAL) in High/Low WHR Males/Females Stress responders (combined cortisol & BP) (Means  $\pm$  SEM)

The stress response group \*gender interaction\*waist-hip ratio group interaction ( $F(1, 51) = 0.510$ ;  $p = 0.479$ ; NS) and stress response group \*gender interaction ( $F(1, 51) = 0.053$ ;  $p = 0.819$ ; NS) failed to reach significance.

A trend for a main effect of stress response group was observed ( $F(1, 51) = 3.243$ ;  $p = 0.078$ ). Stress responders demonstrated poorer performance (fewer correct trials) on the PAL task compared with non-responders ( $8.35 \pm 2.52$  and  $14.09 \pm 1.96$  words respectively).

#### (d) Pattern Recognition Memory (PRM)

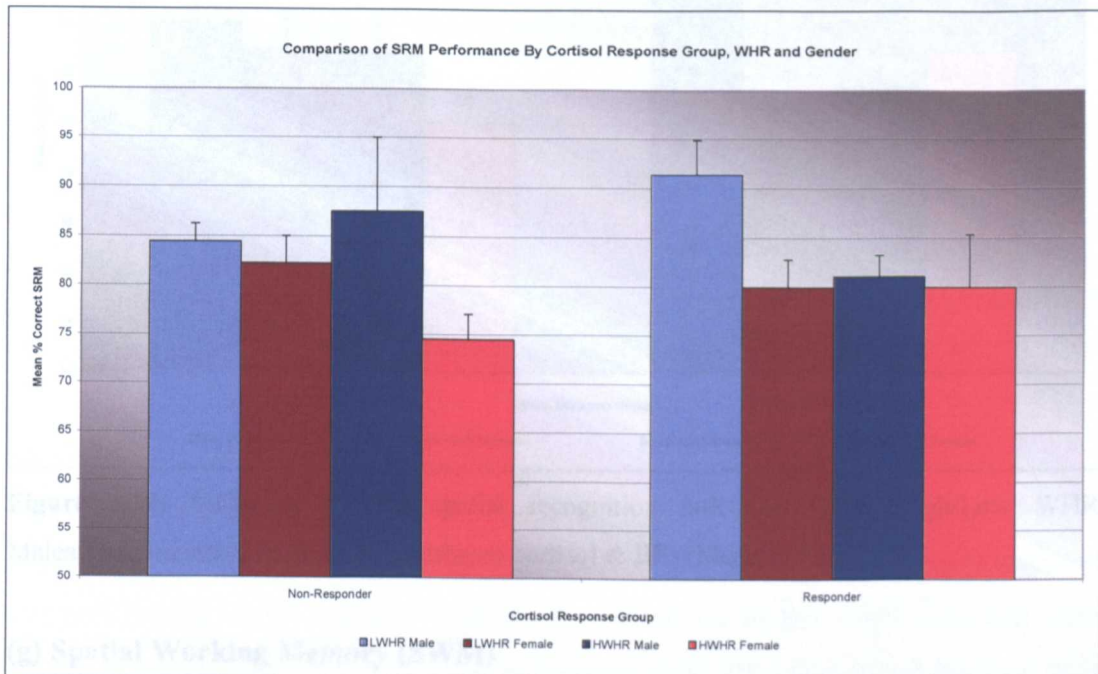
No significant interactions or main effects of response group (stress or cortisol), waist-hip ratio or gender were observed.

#### (e) Rapid Visual Processing (RVP)

No significant interactions or main effects of response group (stress or cortisol), waist-hip ratio or gender were observed.

**(f) Spatial Recognition Memory (SRM)****- Cortisol Response Group**

A significant cortisol response group\*waist-hip ratio group\*gender interaction was observed ( $F(1, 51) = 6.403$ ;  $p < 0.05$ ). Post hoc analyses revealed that male low waist-hip ratio responders recognised significantly more spatial locations than male high waist-hip ratio responders ( $p = 0.003$ ) ( $91.43\% \pm 1.80$  and  $81.11\% \pm 2.17$  respectively). Male low waist-hip ratio responders also performed significantly better than female low waist-hip ratio responders in the same task ( $91.43\% \pm 1.80$  and  $80\% \pm 2.78$  respectively) ( $p = 0.008$ ). This is illustrated in Figure 6.21 below.



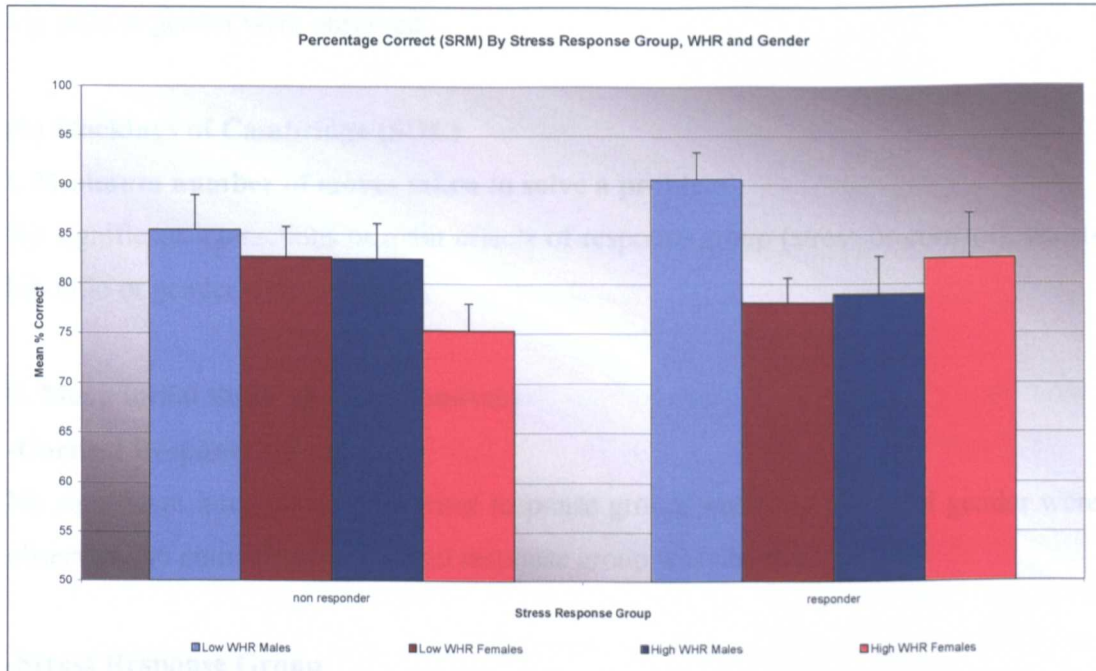
**Figure 6.21:** %Correct on the spatial recognition task (SRM) in High/Low WHR Males/Females cortisol responders (Means ± SEM)

A trend for a cortisol response group\*gender interaction was observed ( $F(1, 51) = 2.294$ ;  $p = 0.133$ ; NS). The cortisol response group \*gender interaction ( $F(1, 51) = 0.294$ ;  $p = 0.590$ ; NS) and cortisol response group\*waist-hip ratio interaction ( $F(1, 51) = 0.179$ ;  $p = 0.674$ ; NS) failed to reach significance. No main effect of cortisol response group was observed ( $F(1, 51) = 0.012$ ;  $p = 0.913$ ).

**- Stress Response Group (Cortisol and BP)**

A significant combined cortisol and BP response group\*waist-hip ratio group\*gender interaction was observed ( $F(1, 51) = 4.841$ ;  $p < 0.05$ ). However, Bonferroni corrected t-tests failed to reach significance. Based on means, low waist-hip ratio male stress

responders recognised significantly more locations than high waist-hip ratio male stress responders ( $90.83 \pm 2.00$  and  $80 \pm 3.87$  respectively) ( $p=0.028$ ). Further, high waist-hip ratio female non-responders demonstrated poorer performance (recognising fewer locations) than low waist-hip female non-responders ( $74.50 \pm 2.63$  and  $82.08 \pm 2.17$  respectively) ( $p=0.036$ ). This is illustrated in Figure 6.21 below.



**Figure 6.22:** %Correct on the spatial recognition task (SRM) in High/Low WHR Males/Females stress responders (combined cortisol & BP) (Means  $\pm$  SEM)

### (g) Spatial Working Memory (SWM)

#### i. Number of Errors

##### - Cortisol Response Group

A trend for a cortisol response group\*gender interaction was observed ( $F(1, 51) = 3.055$ ;  $p=0.087$ ). Post hoc analyses revealed a significant difference between male and female cortisol non-responders for the number of errors made across trials. Female cortisol non-responders made significantly more errors compared with males of the same group ( $26.84 \pm 3.89$  and  $9.00 \pm 3.40$  respectively) ( $p=0.004$ ).

The cortisol response group \*gender interaction\*waist-hip ratio group interaction ( $F(1, 51) = 0.098$ ;  $p=0.756$ ; NS) and cortisol response group \*waist-hip ratio interaction ( $F(1, 51) = 0.321$ ;  $p=0.573$ ; NS) failed to reach significance. No main effect of cortisol response group was observed ( $F(1, 51) = 1.108$ ;  $p=0.298$ ; NS).

**-Stress Response Group**

No significant interactions of stress response group, waist-hip ratio and gender were observed. No main effect of stress response group was observed.

**ii. Strategy**

No significant interactions or main effects of response group (stress or cortisol), waist-hip ratio or gender were observed.

**(h) Stockings of Cambridge (SOC)****i. Minimum number of moves taken to solve a problem**

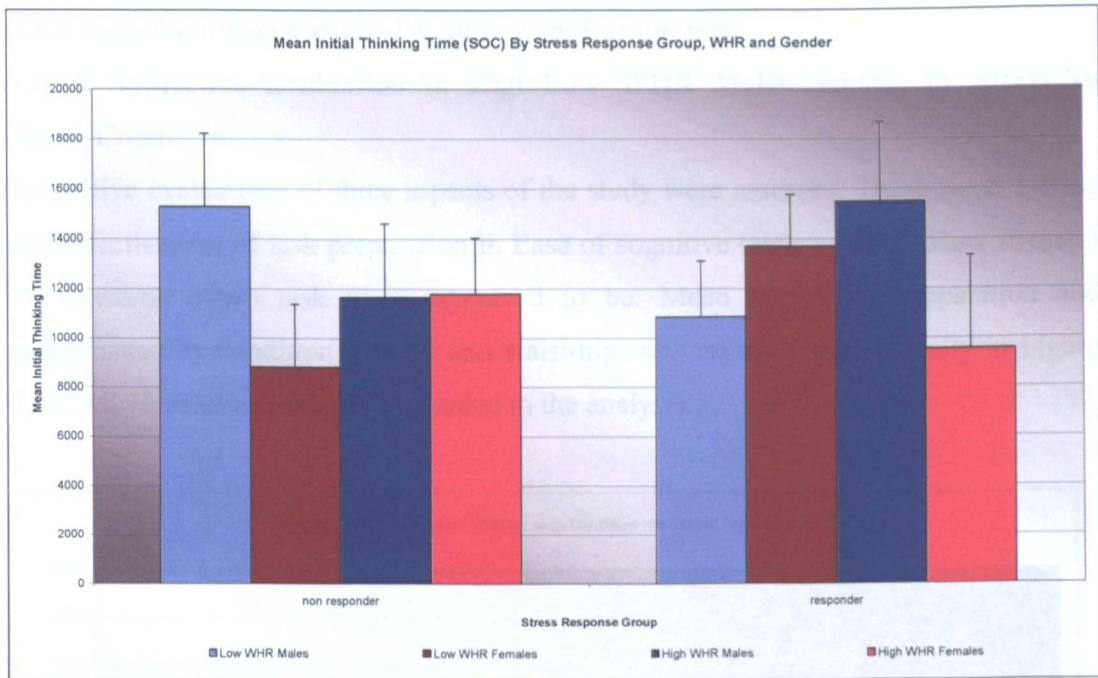
No significant interactions or main effects of response group (stress or cortisol), waist-hip ratio or gender were observed.

**ii. Mean initial thinking time (5 moves)****-Cortisol Response Group**

No significant interactions of cortisol response group, waist-hip ratio and gender were observed. No main effect of cortisol response group was observed.

**-Stress Response Group**

The stress response group\*waist-hip ratio group\*gender interaction just failed to reach significance ( $F(1, 51) = 3.929; p = 0.053$ ). However, post hoc analysis using Bonferroni corrected t-tests did not reach significance. Based on means, high waist-hip ratio female non-responders had longer initial thinking time for a five-move problem than low waist-hip ratio female non-responders ( $11316 \pm 1196$  and  $8285 \pm 1056$  respectively). While the opposite pattern occurred in male non-responders. Low waist-hip ratio male non-responders took longer than other non-responders and than low waist-hip ratio male responders. Low waist-hip ratio female non-responders were faster than male low waist-hip ratio non-responders and female low waist-hip ratio responders. Further, high waist-hip ratio female non-responders took a longer initial thinking time for a five-move problem than high waist-hip ratio female responders ( $11316 \pm 1196$  and  $7475 \pm 1411$  respectively). This is illustrated in Figure 6.23 below.



**Figure 6.23:** Mean Initial Thinking time on the Stockings of Cambridge Task (SOC) in High/Low WHR Males/Females stress responders (combined cortisol & BP) (Means  $\pm$  SEM)

The stress response group \*waist-hip ratio interaction ( $F(1, 51) = 0.021$ ;  $p = 0.886$ ; NS) and the stress response group \*gender interaction ( $F(1, 51) = 0.176$ ;  $p = 0.677$ ; NS) failed to reach significance. No main effect of cortisol response group was observed ( $F(1, 51) = 0.069$ ;  $p = 0.793$  NS).

### iii. Mean subsequent thinking time (following the first move in a 5-move problem)

No significant interactions or main effects of response group (stress or cortisol), waist-hip ratio or gender were observed.

#### 1. Evaluation of Preparation

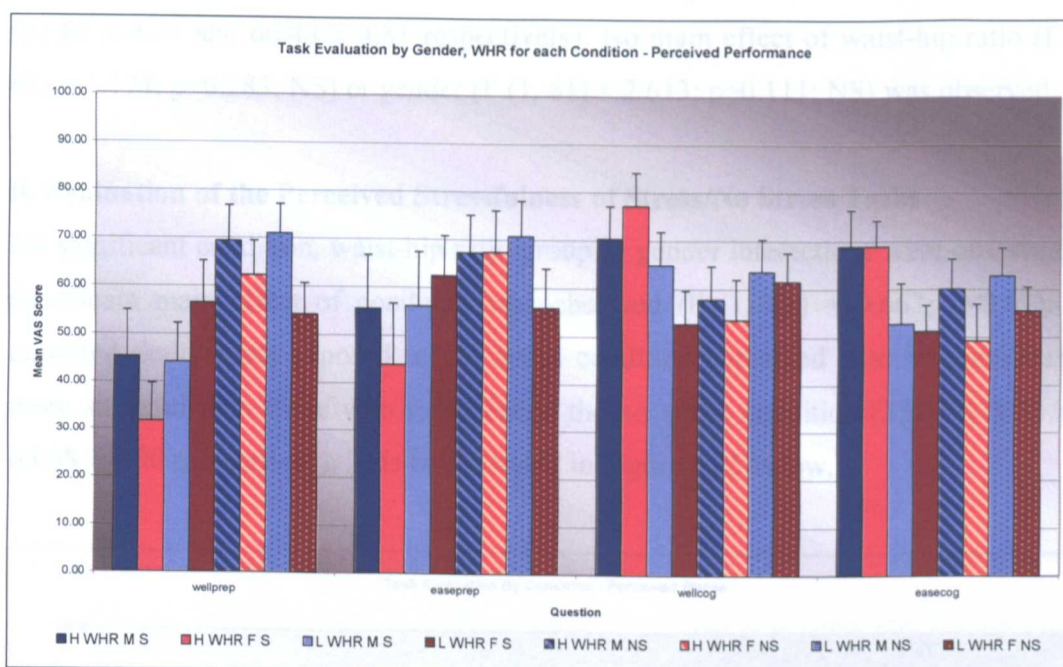
##### - Effectiveness of Preparation

No significant interactions of condition, waist-hip ratio group or gender were observed. The main effect of condition on subjective perception of how well prepared the individual was significant ( $F(1, 61) = 12.294$ ;  $p < 0.01$ ). Preparation was evaluated as being poorer in the stress condition than in the no stress condition ( $55.16 \pm 3.81$  and  $36.13 \pm 3.86$  respectively). No main effect of waist-hip ratio ( $F(1, 61) = 0.067$ ;  $p = 0.796$ ; NS) or gender ( $F(1, 61) = 0.799$ ;  $p = 0.375$ ; NS) was observed.

### 6.4.3 Associated Psychological & Biological Parameters

#### 6.4.3.1 Subjective Evaluation in High/Low WHR Males/Females by Stress/No Stress Condition

Subjective evaluations of three aspects of the study were assessed. These were; i. Ease and effectiveness of task preparation ii. Ease of cognitive testing and iii. How stressful the stress/no stress task were perceived to be. Mean scores for preparation and performance by condition, gender and waist-hip ratio are shown graphically in Figure 6.24. All 70 observations were included in the analysis.



**Figure 6.24:** Subjective Task Evaluation of the stress & cognitive tasks in High/Low WHR Males/Females by Stress/No Stress Condition (Means  $\pm$  SEM).

#### i. Evaluation of Preparation

##### - Effectiveness of Preparation

No significant interactions of condition, waist-hip ratio group or gender were observed. The main effect of condition on subjective perception of how well prepared the individual was significant ( $F(1, 61) = 12.294$ ;  $p < 0.01$ ). Preparation was evaluated as being poorer in the stress condition than in the no stress condition ( $55.16 \pm 3.81$  and  $36.13 \pm 3.86$  respectively). No main effect of waist-hip ratio ( $F(1, 61) = 0.067$ ;  $p = 0.796$ ; NS) or gender ( $F(1, 61) = 0.799$ ;  $p = 0.375$ ; NS) was observed.

**Figure 6.25:** Subjective Task Evaluation of the Stress/No Stress & Cognitive tasks (Perceived Stress) Females by Stress/No Stress Condition (Means  $\pm$  SEM)

### - Ease of Preparation

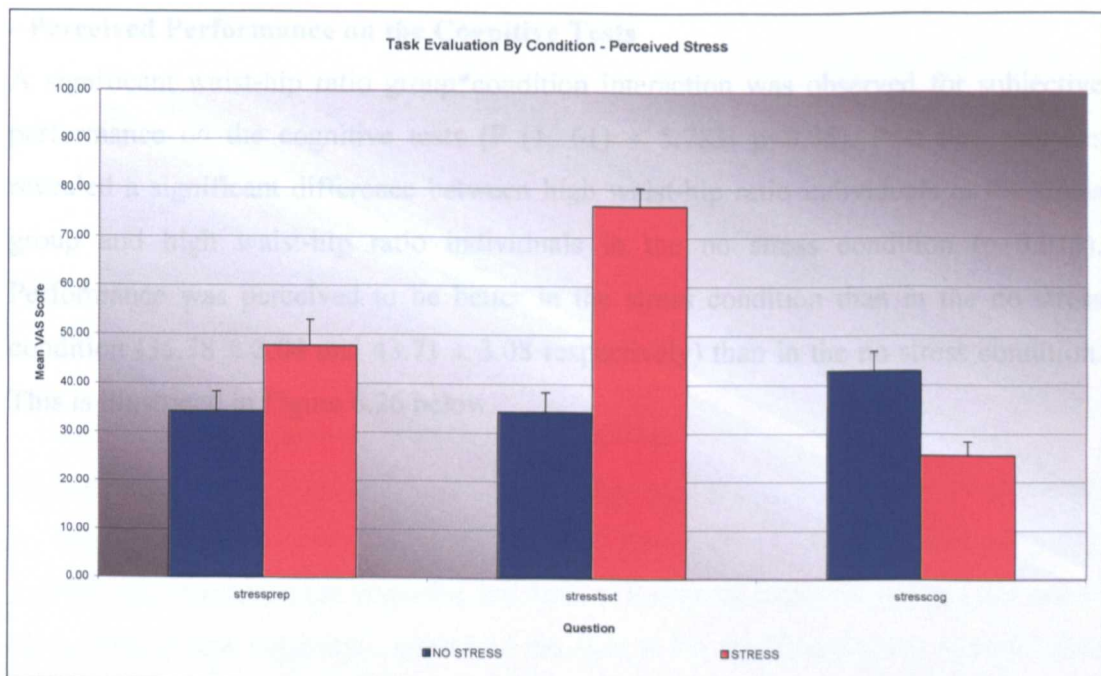
No significant interactions or main effects of condition, waist-hip ratio or gender were observed. The same pattern as for preparedness is evident in Figure 6.24.

### - Stressfulness of Preparation

No significant interaction of condition, waist-hip ratio group or gender were observed. A significant main effect of condition was observed for reported perceived stressfulness of the preparation period ( $F(1, 61) = 4.670$ ;  $p < 0.05$ ). Preparation was evaluated as being significantly more stressful in the stress condition compared with the no stress condition ( $51.81 \pm 4.74$  and  $66.43 \pm 4.81$  respectively). No main effect of waist-hip ratio ( $F(1, 61) = 1.174$ ;  $p = 0.283$ ; NS) or gender ( $F(1, 61) = 2.613$ ;  $p = 0.111$ ; NS) was observed.

### ii. Evaluation of the Perceived Stressfulness of Stress/No Stress Tasks

No significant condition, waist-hip ratio group or gender interactions were observed. A significant main effect of condition was observed ( $F(1, 61) = 59.663$ ;  $p < 0.01$ ). As expected, individuals exposed to the stress condition perceived it to be significantly more stressful than those who experienced the no stress condition ( $23.02 \pm 3.94$  and  $66.38 \pm 4.00$  respectively). This is illustrated in Figure 6.25 below.



**Figure 6.25:** Subjective Task Evaluation of the Stress/No Stress & Cognitive tasks (Perceived Stress) Females by Stress/No Stress Condition (Means  $\pm$  SEM)

No main effect of waist-hip ratio ( $F(1, 61) = 0.045$ ;  $p=0.832$ ; NS) or gender ( $F(1, 61) = 2.181$ ;  $p=0.145$ ; NS) was observed.

### **iii. Evaluation of Cognitive Testing and Perceived Performance**

#### **- Ease of Cognitive Tests**

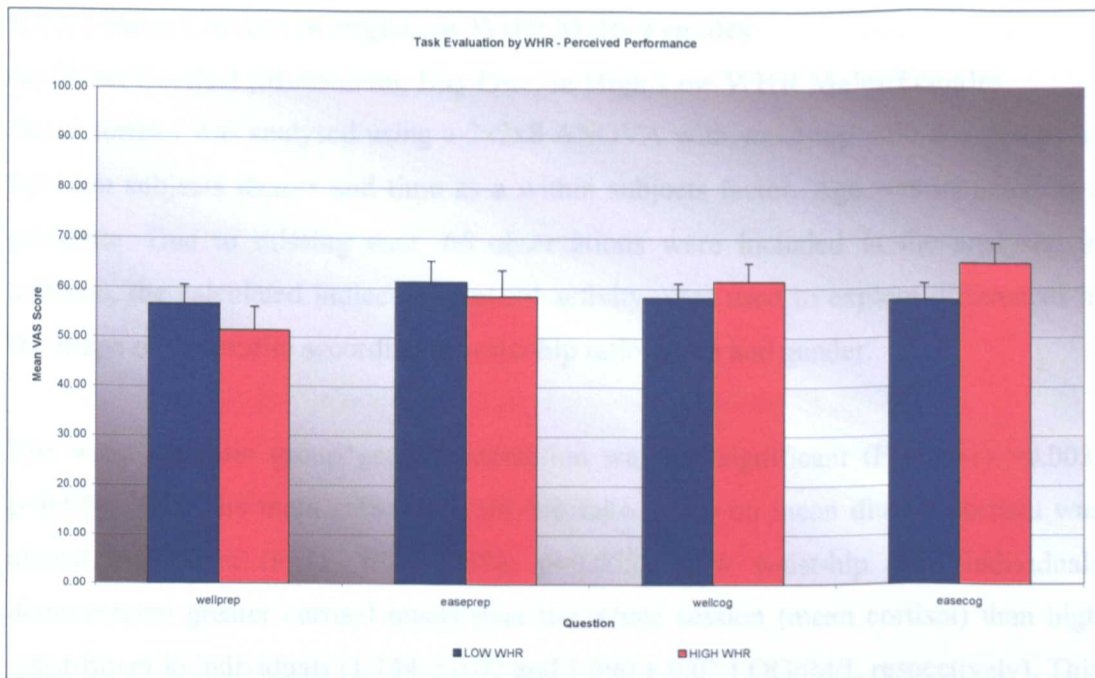
A significant condition\*waist-hip ratio group also was observed ( $F(1, 61) = 8.335$ ;  $p<0.01$ ). Post hoc analyses revealed that high waist-hip ratio individuals in the stress condition perceived the cognitive tests to be significantly easier than low waist-hip ratio individuals in the same condition ( $p=0.009$ ) ( $26.94 \pm 5.09$  and  $47.26 \pm 5.21$  respectively). The condition\*gender\*waist-hip ratio group interaction ( $F(1, 61) = 0.733$ ;  $p=0.395$ ; NS) and condition\*gender interaction ( $F(1, 61) = 1.019$ ;  $p=0.317$ ; NS) failed to reach significance.

A main effect of waist-hip ratio was observed ( $F(1, 61) = 3.811$ ;  $p<0.05$ ). High waist-hip ratio individuals reported greater subjective ease in completing the cognitive tests than low waist-hip ratio individuals ( $33.82 \pm 3.75$  and  $43.72 \pm 3.34$  respectively). No main effect of condition ( $F(1, 61) = 0.661$ ;  $p=0.419$ ; NS) or gender ( $F(1, 61) = 0.071$ ;  $p=0.790$ ; NS) was observed.

#### **- Perceived Performance on the Cognitive Tests**

A significant waist-hip ratio group\*condition interaction was observed for subjective performance on the cognitive tests ( $F(1, 61) = 5.783$ ;  $p<0.05$ ). Post hoc analyses revealed a significant difference between high waist-hip ratio individuals in the stress group and high waist-hip ratio individuals in the no stress condition ( $p=0.018$ ). Performance was perceived to be better in the stress condition than in the no stress condition ( $35.78 \pm 3.04$  and  $43.71 \pm 3.08$  respectively) than in the no stress condition. This is illustrated in Figure 6.26 below.





**Figure 6.26:** Subjective Task Evaluation of the Stress/No Stress & Cognitive tasks (Performance) in High/Low WHR (Means  $\pm$  SEM)

The condition\*gender\*waist-hip ratio group interaction ( $F(1, 61) = 0.891$ ;  $p = 0.349$  NS) and condition\*gender interaction ( $F(1, 61) = 0.108$ ;  $p = 0.744$ ; NS) failed to reach significance.

A trend for a main effect of condition on perceived performance on the cognitive tasks was observed ( $p = 0.072$ ). Individuals in the stress condition perceived their performance on the cognitive tests to be better than those in the no stress condition ( $35.78 \pm 3.04$  and  $43.71 \pm 3.08$  respectively). This effect is possibly due to the waist-hip ratio\*condition interaction described earlier. No main effect of waist-hip ratio ( $F(1, 61) = 1.424$ ;  $p = 0.237$ ; NS) or gender ( $F(1, 61) = 2.373$ ;  $p = 0.129$ ; NS) was observed.

#### - Perceived Stressfulness of the Cognitive Testing

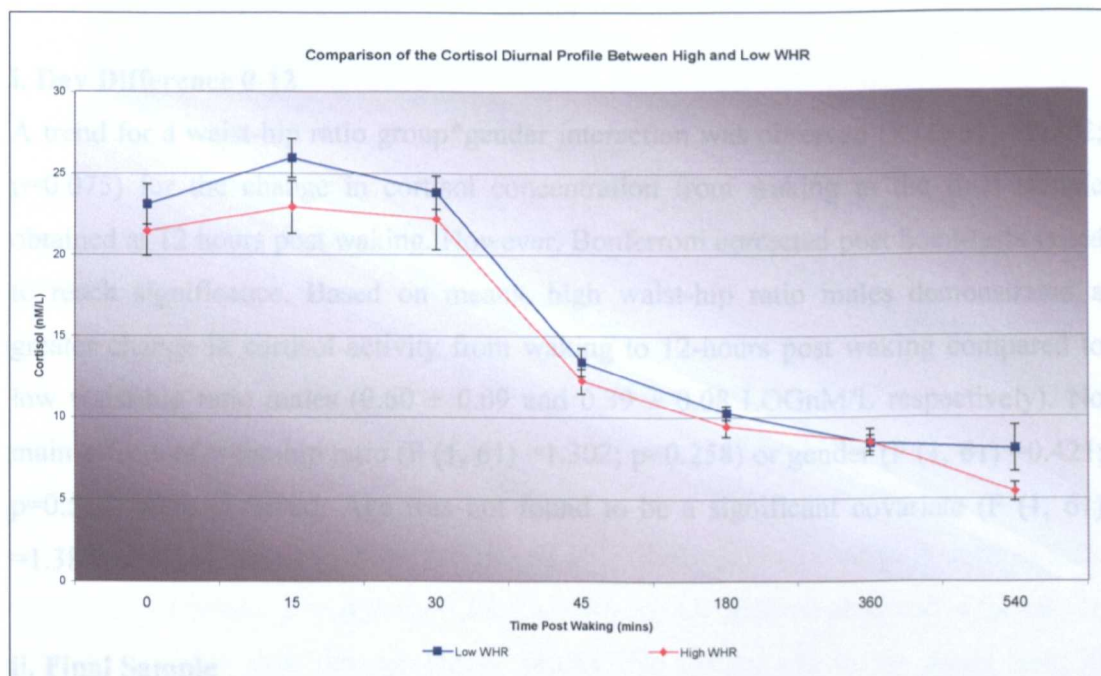
No significant interactions of condition, waist-hip ratio group or gender were observed. A significant main effect of condition was found ( $F(1, 61) = 13.274$ ;  $p < 0.01$ ). Individuals completing the cognitive test battery following exposure to the Trier Social Stress Test (stress condition), perceived the tests to be significantly less stressful than those in the no stress condition ( $75.56 \pm 3.53$  and  $57.22 \pm 3.58$  respectively). No main effect of waist-hip ratio ( $F(1, 61) = 1.492$ ;  $p = 0.227$ ; NS) or gender ( $F(1, 61) = 0.012$ ;  $p = 0.915$ ; NS) was observed.

### 6.4.3.2 Basal Cortisol in High/Low WHR Males/Females

#### (a) Basal Cortisol (Monitoring Day One) in High/Low WHR Males/Females

Basal cortisol was analysed using a 2x2x8 ANOVA with waist-hip ratio and gender as between subjects factors and time as a within subjects factor. Age was included as a covariate. Due to missing data, 66 observations were included in the analysis. In addition, the calculated indices of cortisol activity were used to explore differences in the shape of the profile according to waist-hip ratio group and gender.

The waist-hip ratio group\*gender interaction was not significant ( $F(1, 61) = 0.003$ ;  $p = 0.958$ ; NS). The main effect of waist-hip ratio group on mean diurnal cortisol was almost significant ( $F(1, 61) = 3.888$ ;  $p = 0.053$ ). Low waist-hip ratio individuals demonstrated greater cortisol levels over the whole session (mean cortisol) than high waist-hip ratio individuals ( $1.144 \pm 0.02$  and  $1.090 \pm 0.02$  LOGnM/L respectively). This is illustrated in Figure 6.27 below.



**Figure 6.27** Basal Diurnal Cortisol Profiles in High/Low WHR (Means ± SEM)

A significant main effect of time on cortisol concentration was observed ( $F(1,427) = 4.873$ ;  $p < 0.01$ ). Pairwise comparisons revealed that the cortisol sample obtained immediately upon waking (time = 0) significantly differed in concentration to all other samples obtained during that monitoring day. Samples at time points 2, 3 and 4 (15min, 30min and 45minutes) differed significantly from all other sample points but not from

each other. Samples 5-8 (3-12 hours at 3h intervals) differed significantly from all remaining samples and from each other (smallest  $p=0.047$ ). As is evident from Figure 6.27. The profile for the high and low waist-hip ratio groups was similar.

No main effect of gender was observed ( $F(1, 61) = 2.289$ ;  $p=0.135$ ; NS). Age was not found to be a significant covariate ( $F(1, 61) = 0.437$ ;  $p=0.551$ ; NS).

### **(b) Analysis of the Diurnal Profile using Calculated Indices of Activity**

Each index was analysed using a 2x2 ANOVA with waist-hip ratio group and gender as between subjects' factors. Age was included as a covariate.

For the following indices; i. Area under the curve (AUC), ii. Area under the curve with reference to the first sample (AURC), iii. Mean Increase (MI), iv. Change 0-30, v. Day Mean, vi. Diurnal Mean, vii. Day Difference 3-12, no significant interactions or main effects of waist-hip ratio or gender were observed.

#### **i. Day Difference 0-12**

A trend for a waist-hip ratio group\*gender interaction was observed ( $F(1, 61) = 3.282$ ;  $p=0.075$ ) for the change in cortisol concentration from waking to the final sample obtained at 12 hours post waking. However, Bonferroni corrected post hoc t-tests failed to reach significance. Based on means, high waist-hip ratio males demonstrated a greater change in cortisol activity from waking to 12-hours post waking compared to low waist-hip ratio males ( $0.60 \pm 0.09$  and  $0.39 \pm 0.08$  LOGnM/L respectively). No main effects of waist-hip ratio ( $F(1, 61) = 1.302$ ;  $p=0.258$ ) or gender ( $F(1, 61) = 0.421$ ;  $p=0.519$ ) were observed. Age was not found to be a significant covariate ( $F(1, 61) = 1.384$ ;  $p=0.244$ ; NS).

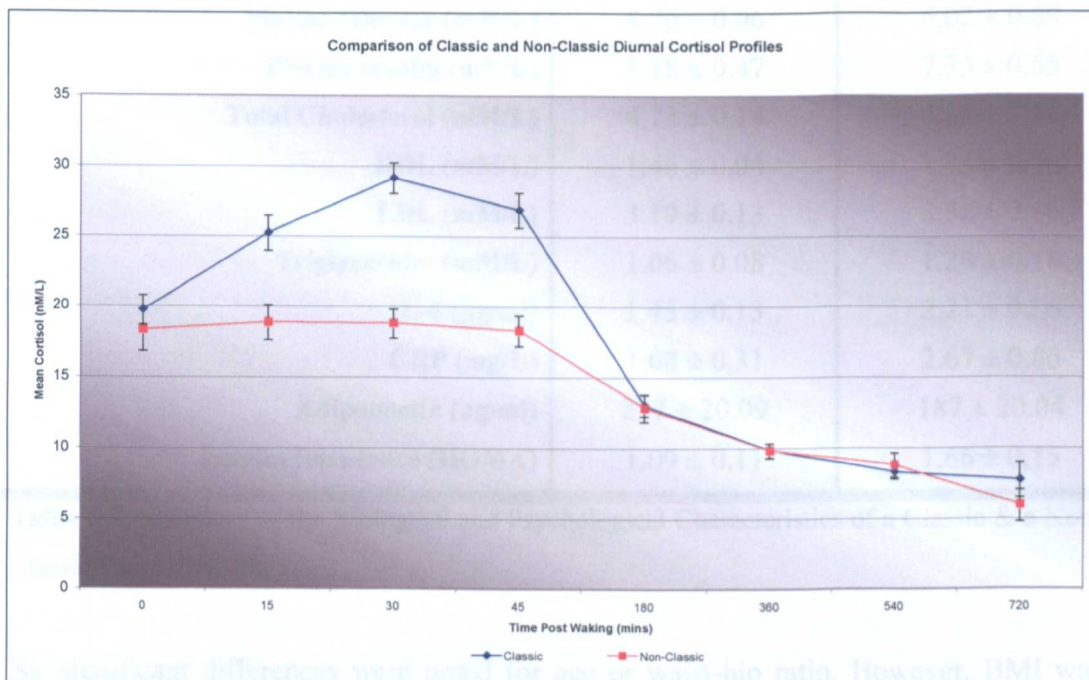
#### **ii. Final Sample**

Analysis of the final sample in the profile (12 hours post waking) revealed a trend for a main effect of waist-hip ratio group ( $F(1, 61) = 3.017$ ;  $p=0.087$ ). Low waist-hip ratio individuals tended to exhibit a greater mean cortisol at 12 hours post waking than high waist-hip ratio individuals ( $0.786 \pm 0.05$  and  $0.656 \pm 0.06$  LOGnM/L respectively). No main effect of gender was observed ( $F(1, 61) = 0.692$ ;  $p=0.409$ ; NS). Age was not found to be a significant covariate ( $F(1, 61) = 1.099$ ;  $p=0.299$ ; NS).

### 6.4.3.3 Classic & Non-Classic Diurnal Cortisol Profiles

#### (a) Forming the profiles

Following classification by three independent observers, as described in Chapter Five, the composite classic and non-classic cortisol diurnal profiles were constructed and are displayed in Figure 6.28 below.



**Figure 6.28:** Basal Diurnal Cortisol Profiles in the Classic/Non-Classic Profile Groups (Means  $\pm$  SEM)

As in Chapter Five, the differences between the profile groups were confirmed statistically by assessment of the cortisol indices. A significantly greater mean increase ( $p=0.021$ ), area under the curve with reference to zero (AUC) ( $p=0.001$ ), area under the curve with reference to the first sample (AURC) ( $p=0.016$ ) and change in cortisol from waking to 30 minutes post waking (Change 0-30) ( $p=0.010$ ) were observed in the classic profile compared with the non-classic profile. No differences in the mean level of cortisol were observed.

A summary of the characteristics explored to compare the 'classic' and 'non-classic' diurnal cortisol profiles is shown in Table 6.7.

Variable	CLASSIC N = 37	NON-CLASSIC N = 28
Waist-Hip Ratio	0.84 ± 0.01	0.87 ± 0.02
Age	47.32 ± 1.21	44.29 ± 1.29
BMI	26.37 ± 0.74	29.78 ± 1.49
Perceived Stress	15.51 ± 1.11	19.18 ± 1.42
Plasma Glucose (mM/L)	4.70 ± 0.06	5.02 ± 0.09
Plasma insulin (mU/L)	5.18 ± 0.47	7.33 ± 0.65
Total Cholesterol (mM/L)	4.72 ± 0.14	4.81 ± 0.19
HDL (mM/L)	1.46 ± 0.04	1.35 ± 0.06
LDL (mM/L)	3.19 ± 0.13	3.32 ± 0.16
Triglycerides (mM/L)	1.06 ± 0.08	1.26 ± 0.18
IL-6 (pg/ml)	1.43 ± 0.13	2.21 ± 0.26
CRP (mg/L)	1.08 ± 0.31	2.67 ± 0.80
Adiponectin (ng/ml)	237 ± 20.09	187 ± 20.04
Insulin Resistance (HOMA)	1.09 ± 0.11	1.66 ± 0.15

**Table 6.7:** Summary of the Biological and Psychological Characteristics of a Classic & a Non-Classic Diurnal Profile

No significant differences were noted for age or waist-hip ratio. However, BMI was significantly higher in the non-classic profile group than the classic ( $t(63) = -2.047$ ;  $p = 0.05$ ). Further, there was a significantly greater reporting of perceived stress in the non-classic group ( $t(63) = -2.064$ ;  $p < 0.05$ ).

In a repeat of the analysis conducted in Chapter Five, the associated metabolic syndrome and obesity related biomarkers are discussed in the following section.

#### **(b) Analysis of relevant Biomarkers in Metabolic Syndrome Symptomology**

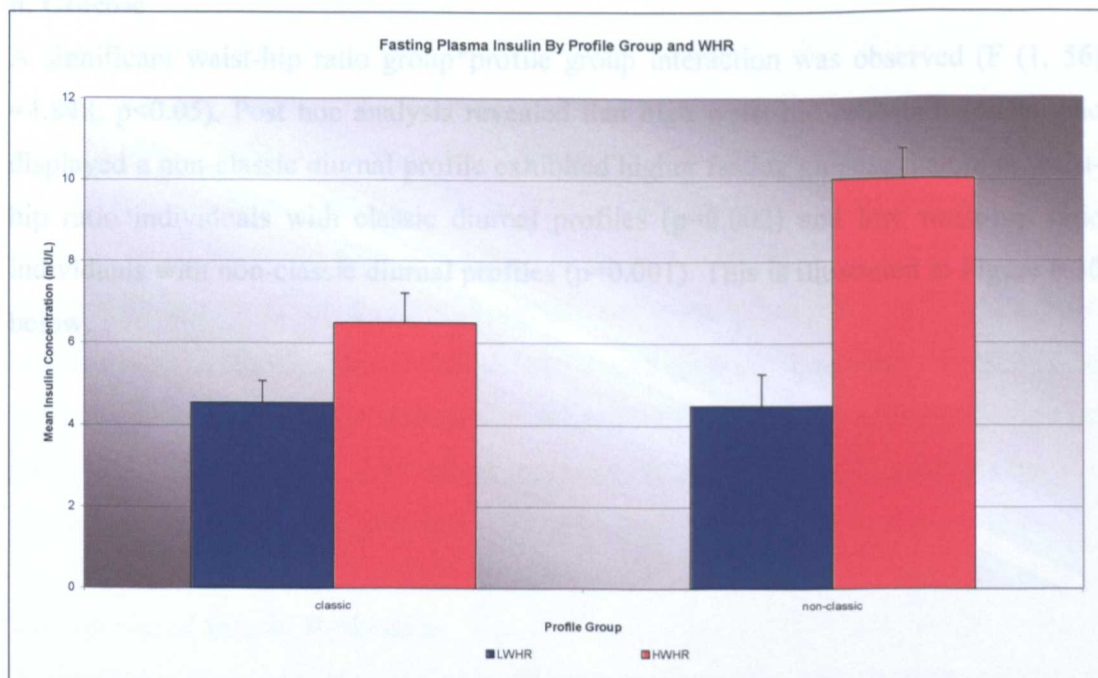
Each of the biomarkers; i. insulin, ii. glucose, iii. cholesterol, iv. triglycerides, v. HDL, vi. LDL, vii. IL-6, viii. CRP, ix. adiponectin was analysed individually using a 2x2x2 ANOVA with waist-hip ratio, gender and profile group as between subjects factors. Mean values for each biomarker by WHR and gender are shown in Table 6.8 below.

Biomarker	Total	LWHR Male	HWRH Male	LWHR Female	HWRH Female
Insulin (mmol/L)	6.46±4.21	4.42±2.71	9.83±6.66	4.76±1.81	8.01±2.71
Glucose (mmol/L)	4.83±0.46	4.80±0.41	4.98±0.30	4.56±0.31	5.12±0.59
Cholesterol (mmol/L)	4.76±0.92	4.24±0.89	4.86±0.92	4.99±0.79	4.90±1.00
HDL (mmol/L)	1.41±0.04	1.31±0.20	1.28±0.26	1.58±0.26	1.40±0.35
LDL (mmol/L)	3.25±0.81	2.83±0.82	3.59±0.93	3.33±0.66	3.26±0.79
Triglycerides (mmol/L)	1.14±0.70	0.91±0.51	1.18±0.39	0.90±0.33	1.68±1.10
CRP (mg/L)	1.78±3.14	0.42±0.57	2.51±3.25	0.72±0.73	4.06±4.95
IL-6 (pg/ml)	1.80±1.53	0.95±0.45	2.48±2.15	1.35±0.60	2.74±1.84
Adiponectin (ng/ml)	220±119	181±21	155±84	298±147	214±77
Insulin Resistance	1.34±0.77	0.99±0.69	1.84±0.98	0.94±0.38	1.82±0.61

**Table 6.8:** Summary of Blood Biomarkers in High/Low WHR Males/Females (Means ± SD)

### i. Insulin

A significant waist-hip ratio group\*profile group interaction was observed ( $F(1, 56) = 6.849; p < 0.05$ ). This is illustrated in Figure 6.29.



**Figure 6.29:** Fasting Plasma Insulin in High/Low WHR males/females & by Classic/Non-Classic Profile Group (Means ± SEM)

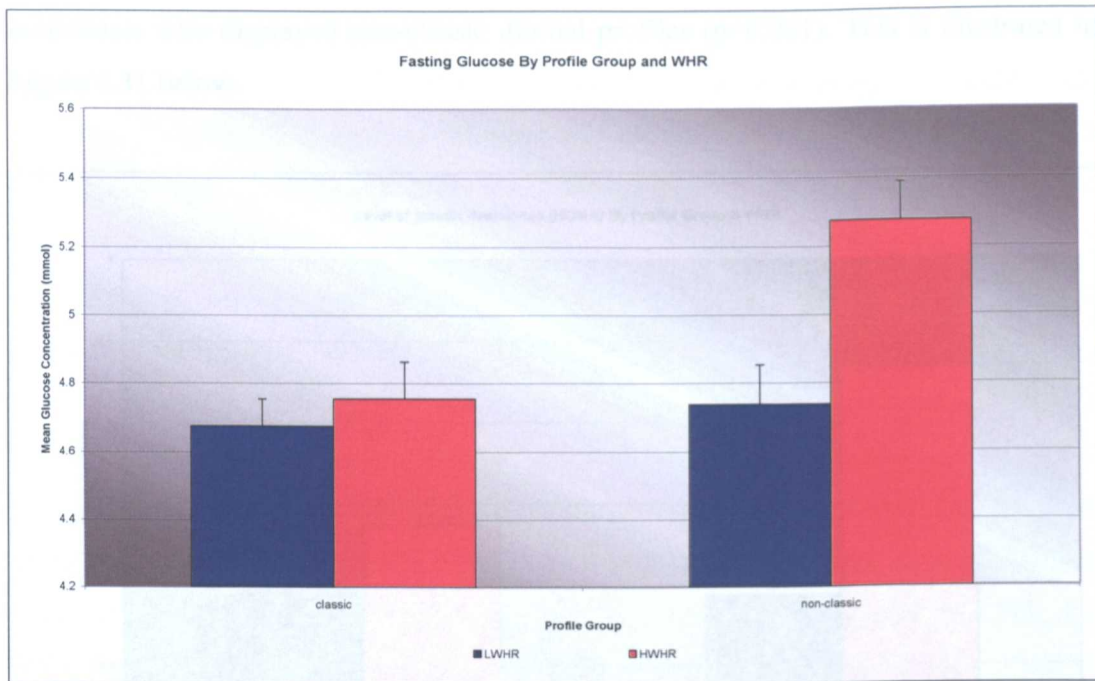
Post hoc analyses revealed that high waist-hip ratio individuals who displayed a non-classic diurnal profile exhibited higher fasting plasma insulin than high waist-hip ratio individuals with a classic diurnal profile ( $p=0.008$ ) and than low waist-hip ratio individuals with a non-classic diurnal profile ( $p=0.001$ ).

The profile group\*waist-hip ratio\*gender interaction ( $F(1, 56) = 1.721$ ;  $p=0.195$ ; NS), profile group\*gender interaction ( $F(1, 56) = 0.582$ ;  $p=0.499$ ; NS) and waist-hip ratio\*gender interaction ( $F(1, 56) = 0.895$ ;  $p=0.348$ ) failed to reach significance.

A main effect of profile group was observed ( $F(1, 56) = 5.919$ ;  $p<0.01$ ). Higher measured fasting plasma insulin was observed in individuals who exhibited a non-classic diurnal cortisol profile than in those who did not ( $7.25 \pm 0.53$  and  $5.51 \pm 0.46$  mU/L respectively). Further, a main effect of waist-hip ratio group on insulin was observed ( $F(1, 59) = 23.357$ ;  $p<0.01$ ). High waist-hip ratio individuals demonstrated higher insulin compared with low waist-hip ratio individuals. No main effect of gender was observed ( $F(1, 56) = 0.192$ ;  $p=0.663$ ; NS).

## **ii. Glucose**

A significant waist-hip ratio group\*profile group interaction was observed ( $F(1, 56) = 4.848$ ;  $p<0.05$ ). Post hoc analysis revealed that high waist-hip ratio individuals who displayed a non-classic diurnal profile exhibited higher fasting glucose than high waist-hip ratio individuals with classic diurnal profiles ( $p=0.002$ ) and low waist-hip ratio individuals with non-classic diurnal profiles ( $p=0.001$ ). This is illustrated in Figure 6.30 below.



**Figure 6.30:** Fasting Glucose in High/Low WHR males/females & by Classic/Non-Classic Profile Group (Means  $\pm$  SEM)

The profile group\*waist-hip ratio\*gender interaction ( $F(1, 56) = 0.081$ ;  $p = 0.777$ ; NS), profile group\*gender interaction ( $F(1, 56) = 0.727$ ;  $p = 0.398$ ; NS) and waist-hip ratio\*gender interaction ( $F(1, 56) = 2.360$ ;  $p = 0.130$ ) failed to reach significance.

The profile group\*waist-hip ratio\*gender interaction ( $F(1, 56) = 1.572$ ;  $p = 0.215$ ; NS). A main effect of waist-hip ratio group on fasting glucose was observed ( $F(1, 59) = 13.268$ ;  $p < 0.01$ ). High waist-hip ratio individuals demonstrated higher observed level of fasted glucose compared with low waist-hip ratio individuals ( $5.02 \pm 0.08$  and  $4.71 \pm 0.07$  mM/L respectively). Further, a significant main effect of profile group was observed ( $F(1, 56) = 7.434$ ;  $p < 0.01$ ). Higher fasted glucose was evident in individuals who exhibited a non-classic diurnal cortisol profile than in those with classic profiles ( $5.01 \pm 0.08$  and  $4.72 \pm 0.07$  mM/L respectively). No main effect of gender was observed ( $F(1, 56) = 1.508$ ;  $p = 0.225$ ; NS).

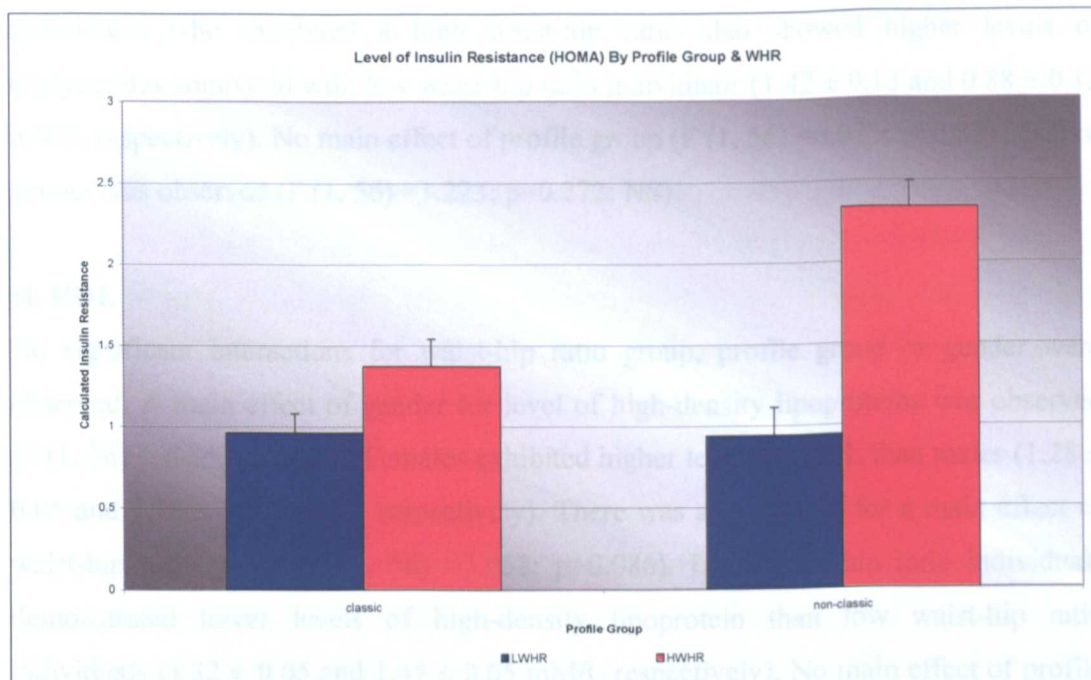
individuals who exhibited classic diurnal profiles ( $1.64 \pm 0.12$  and  $1.17 \pm 0.10$  mmol/L respectively). The profile group\*gender interaction was observed ( $F(1, 56) = 0.411$ ;  $p = 0.514$ ; NS).

### iii. Calculated Insulin Resistance

A significant waist-hip ratio group\*profile group interaction was observed ( $F(1, 56) = 8.581$ ;  $p < 0.05$ ). Post hoc analyses revealed that high waist-hip ratio individuals with non-classic diurnal profiles exhibited greater insulin resistance than high waist-hip ratio individuals who displayed classic diurnal profiles ( $p = 0.001$ ) and low waist-hip ratio



individuals who displayed non-classic diurnal profiles ( $p=0.001$ ). This is illustrated in Figure 6.31 below.



**Figure 6.31:** Insulin Resistance in High/Low WHR males/females & by Classic/Non-Classic Profile Group (Means  $\pm$  SEM)

The profile group\*waist-hip ratio\*gender interaction ( $F(1, 56) = 1.572$ ;  $p=0.215$ ; NS), profile group\*gender interaction ( $F(1, 56) = 0.446$ ;  $p=0.507$ ; NS) and waist-hip ratio\*gender interaction ( $F(1, 56) = 0.528$ ;  $p=0.471$ ) failed to reach significance.

A significant main effect of waist-hip ratio was observed ( $F(1, 58) = 24.687$ ;  $p<0.01$ ). Individuals with a high waist-hip ratio demonstrated greater insulin resistance compared to low waist-hip ratio individuals ( $1.85 \pm 0.12$  and  $0.95 \pm 0.11$  respectively). Further, a main effect of profile group was observed ( $F(1, 56) = 7.434$ ;  $p<0.01$ ). Insulin resistance was greater in individuals who exhibited non-classic diurnal cortisol profiles than in individuals who exhibited classic diurnal profiles ( $1.64 \pm 0.12$  and  $1.17 \pm 0.10$  respectively). No main effect of gender was observed ( $F(1, 56) = 0.431$ ;  $p=0.514$ ; NS).

#### iv. Cholesterol

No significant interactions or main effects of waist-hip ratio group, profile group or gender were observed.

**v. Triglycerides**

No significant interactions for waist-hip ratio group, profile group or gender were observed. A main effect of waist-hip ratio was observed ( $F(1, 59) = 9.816$ ;  $p < 0.01$ ). Individuals who exhibited a high waist-hip ratio also showed higher levels of triglycerides compared with low waist-hip ratio individuals ( $1.42 \pm 0.13$  and  $0.88 \pm 0.12$  mM/L respectively). No main effect of profile group ( $F(1, 56) = 0.023$ ;  $p = 0.881$ ; NS) or gender was observed ( $F(1, 56) = 1.223$ ;  $p = 0.272$ ; NS).

**vi. HDL**

No significant interactions for waist-hip ratio group, profile group or gender were observed. A main effect of gender for level of high-density lipoproteins was observed ( $F(1, 56) = 6.853$ ;  $p < 0.05$ ). Females exhibited higher levels of HDL than males ( $1.28 \pm 0.05$  and  $1.49 \pm 0.05$  mM/L respectively). There was also a trend for a main effect of waist-hip ratio group ( $F(1, 56) = 3.058$ ;  $p = 0.086$ ). High waist-hip ratio individuals demonstrated lower levels of high-density lipoprotein than low waist-hip ratio individuals ( $1.32 \pm 0.05$  and  $1.45 \pm 0.05$  mM/L respectively). No main effect of profile group was observed ( $F(1, 56) = 2.633$ ;  $p = 2.633$ ;  $p = 0.110$ ; NS).

**vii. LDL**

No significant interactions or main effects of waist-hip ratio group, profile group or gender were observed.

**viii. IL-6**

A significant waist-hip ratio group\*profile group interaction was observed ( $F(1, 56) = 4.753$ ;  $p < 0.05$ ). Post hoc analysis revealed that high waist-hip ratio individuals who displayed non-classic diurnal profiles exhibited significantly higher IL-6 than low waist-hip ratio individuals who displayed non-classic diurnal profiles ( $p = 0.004$ ). The profile group\*waist-hip ratio\*gender interaction ( $F(1, 56) = 1.207$ ;  $p = 0.277$ ; NS), profile group\*gender interaction ( $F(1, 56) = 0.790$ ;  $p = 0.378$ ; NS) and waist-hip ratio\*gender interaction ( $F(1, 56) = 0.124$ ;  $p = 0.726$ ) failed to reach significance.

A significant main effect of waist-hip ratio group was observed ( $F(1, 59) = 16.011$ ;  $p < 0.01$ ). Individuals who exhibited a high waist-hip ratio demonstrated higher levels of interleukin-6 than low waist-hip ratio individuals ( $2.54 \pm 0.25$  and  $1.13 \pm 0.23$  pg/ml).

respectively). Further, a trend for a main effect of profile group was observed ( $p=0.086$ ). Higher IL-6 was observed in individuals who exhibited non-classic diurnal cortisol profiles compared to those who did not ( $2.13 \pm 0.25$  and  $1.54 \pm 0.22$  pg/ml respectively). No main effect of gender was observed ( $F(1, 56) = 1.298$ ;  $p=0.260$ ; NS).

#### **ix. CRP**

No significant interactions for waist-hip ratio group, profile group or gender were observed. A main effect of waist-hip ratio was observed ( $F(1, 59) = 13.048$ ;  $p < 0.01$ ). Individuals with a high waist-hip ratio demonstrated higher levels of CRP than low waist-hip ratio individuals ( $3.24 \pm 0.57$  and  $0.50 \pm 0.52$  mg/ml respectively).

#### **x. Adiponectin**

No significant interactions for waist-hip ratio group, profile group or gender were observed. A main effect of waist-hip ratio group for level of adiponectin was observed ( $F(1, 59) = 6.297$ ;  $p < 0.05$ ). High waist-hip ratio individuals exhibited lower levels of adiponectin than low waist-hip ratio individuals ( $172 \pm 20.48$  and  $227 \pm 18.63$  ng/ml respectively). A main effect of gender was also observed ( $F(1, 59) = 11.967$ ;  $p < 0.05$ ). Males exhibited significantly lower levels of adiponectin compared with females ( $150 \pm 20.69$  and  $249 \pm 17.75$  ng/ml respectively).

#### 6.4.4 Summary of Results

- **Stress Responsivity in High/Low Waist-Hip Ratio Males and Females**

High waist-hip ratio males secreted most cortisol in response to the stressor task and exhibited greater cortisol at baseline. When considering change from baseline response to the stressor, low waist-hip ratio males demonstrated the greatest cortisol response (evidenced by a greater percentage rise). Less recovery was observed in the high waist-hip ratio males than in low waist-hip ratio males who demonstrated good HPA regulation (evidenced by cortisol changes post task). High waist-hip ratio females demonstrated the smallest change in cortisol in response to the stressor task. Changes in systolic and diastolic blood pressure generally mirrored changes in cortisol in response to the stress/ no stress interventions. Mean blood pressure responses were highest in high waist-hip ratio males and lowest in low waist-hip ratio females.

- **Cognitive Performance by Stress/No Stress Condition and Stress Responses**

Exposure to the TSST and stress responses (cortisol and blood pressure) impaired performance on the AVLTL compared with no stress exposure. Performance on the paired associates learning task was impaired in high waist-hip ratio males who demonstrated a cortisol and/or cortisol and BP response to the stressor/no stressor task. Performance on the spatial recognition memory task was impaired in high waist-hip ratio individuals generally. Further, impairment was evident in high waist-hip ratio males who demonstrated a cortisol and/or cortisol and BP response to the stressor task.

- **Basal Cortisol Profiles in High/Low Waist-Hip Ratio Males and Females**

Basal cortisol differed in terms of the overall mean cortisol secreted across the diurnal profile according to waist-hip ratio group. High waist-hip ratio individuals secreted less cortisol. No changes in the shape of the profile between high and low waist-hip ratio groups were evident. Further, significantly higher insulin, glucose and insulin resistance were associated with a non-classic diurnal profile. A trend for a higher concentration of IL-6 in non-classic profiles was observed.

## 6.5 Discussion

### 6.5.1 General Aims

This study was based on previous research findings, which proposed that cortisol responses in high waist-hip ratio individuals are elevated compared to low waist-hip ratio individuals. Further, the study explored the possibility of enhanced vulnerability to display cognitive impairment on hippocampal related tasks in those with central obesity. It has already been established that those with central obesity can show cognitive impairment due to metabolic factors such as hypertension, insulin resistance and risk of diabetes (Bent et al., 2000; Elias et al., 2005; Hiltunen et al., 2001). However, this study specifically explored cognitive impairment related to stress exposure, which produced an elevation in salivary cortisol.

### 6.5.2 Stress Responsivity in High/Low WHR Males/Females

#### 6.5.2.1 Physiological & Psychological Responses to Stress

Analysis of cortisol responses to the stress condition suggested that high waist-hip ratio males secreted the most cortisol in response to the stressor. However, high waist-hip ratio males exhibited greater cortisol at baseline prior to stress exposure although this was not significant. Therefore, when the change in cortisol from baseline was considered, high waist-hip ratio males did not demonstrate the greatest response. The change from baseline analysis indicated that *low* waist-hip ratio males exhibited the largest cortisol response to the stressor. Further, blood pressure responses were more elevated in males and tended to be higher in individuals with a high waist-hip ratio. Subjective anxiety as measured by the State Trait Anxiety Inventory was elevated by the stress condition compared with the no stress condition. Self-esteem also reduced because of stress exposure although this did not reach significance. Females, in general and high waist-hip ratio females in particular reported greater anxiety compared to males.

The literature reviewed in Chapter One largely suggested that individuals with central obesity should exhibit a more pronounced cortisol response to a psychosocial stressor. The current findings generally support this. However, the study found heightened stress responsivity occurred in males, whereas previous studies have demonstrated elevated stress responses only in high waist-hip ratio females often only considering females and

not males (Epel et al., 2000; Marin et al., 1992; Moyer et al., 1994). Research exploring stress responsivity in high waist-hip ratio males is sparse and so it cannot be ascertained if the current findings are consistent with previous research. Further, whether high waist-hip ratio males are most responsive to stress must be viewed tentatively due to the observation that these high waist-hip ratio males also demonstrated greater cortisol at baseline and the sample size of this subgroup was low. Mean cortisol over the test session was elevated in high waist-hip ratio males, while low waist-hip ratio males demonstrated a larger response. However, it is important to note that the greater observed concentration of cortisol at baseline conflicts with previous findings in Chapter Five of this thesis, which suggested that basal cortisol, is reduced in high waist-hip ratio individuals. With this in mind, a separate analysis of the cortisol response to the stressor was conducted as a change from a comparable sample time point on Monitoring Day One prior to the test session. This supported the suggestion that the high waist-hip ratio males were indeed the most responsive in the sample. A graphical representation of this can be found in the appendix (Appendix 22).

The observation that males are more responsive to a stressor than females is consistent with previous research (Kirschbaum et al., 1995; Wolf et al., 2001). Perhaps the most feasible explanation for this finding is that the observed effect is a gender difference, not related to waist-hip ratio group (or only minimally associated with waist-hip ratio). This could explain the consistency of findings that suggest that males particularly those with high waist-hip ratios are more responsive. It also would account for the apparent elevated response of the low waist-hip ratio males.

Wolf et al. (2001) reported more pronounced stress responses in males than in females when exploring the effect of stress induced glucocorticoids on cognitive function. No heightened responsivity was observed in females, which is consistent with the current study. To explain the lack of responsivity in females, Wolf et al. (2001) proposed that females are in some way protected from over-responding and hence, males appear more responsive. It has been suggested that oestrogen is protective against the negative effects of stress (Galea et al., 1997). Further, Kudielka et al. (1999) found that estradiol treatment for 2 weeks resulted in a blunted response to stress. However, in animal studies, a potential oestrogenic enhancement of the HPA axis was supported by elevated responses to stress in oestrogen supplemented animals (Burgess and Handa, 1992; Viau

and Meaney, 1991). In humans, the same has been observed. Kirschbaum et al. (1996) exposed a sample of males to short-term estradiol treatment and found enhanced stress responsivity. This contradicts the proposal that oestrogen is protective rather than stimulatory. The idea that sex hormones could be protective against chronic stress may explain the current findings. However, since oestrogen was not assessed in females in this sample this remains suggestive. Therefore, further research is required.

Further, in relation to the role of oestrogen, the level of corticosteroid binding globulin (CBG) can also potentially influence stress responsivity. CBG is bound to the majority of circulating cortisol and has been shown to be higher in pre-menopausal females than in males of the same age (Fernandez-Real et al., 2003) and is higher during oral contraceptive use (Fujimoto et al., 1986; Weigatz et al., 2003). Higher CBG would signal reduced bioavailability of free cortisol and result in dampened responses. Females currently using oral contraceptives were not excluded from participating in the current study since research conflicts over the interaction between oral contraceptive use and cortisol activity. For example, Kirschbaum et al. (1995) failed to find any association between oral contraceptive use and stress responsivity. However, some other studies have found that oral contraceptive use can attenuate the response to stress (Kirschbaum et al., 1999). In the current study, only a small number of females reported oral contraceptive use and none were outliers. It is therefore unlikely to have influenced the pattern of results. It is clear that a number of additional factors, outside the scope of this thesis, need to be assessed in order to determine the true existence of a gender difference. Oestrogen is fat-soluble and can disrupt the feedback of the HPA axis in obesity. Oestrogen levels may be elevated by obesity and central adiposity and hence, could influence hormone levels. It therefore, remains to be explored what the effect of oestrogen and central obesity has on stress responsivity.

Measurement of the response to stress was not limited to changes in cortisol activity. Measures of blood pressure confirmed prior patterns of cortisol activity with greater elevation of blood pressure (both systolic and diastolic) in the stress condition than in the no stress condition. This supports the observation that the Trier Social Stress Test was successful in inducing a stress response marked by both cortisol and blood pressure responses consistent with previous research (Kirschbaum et al., 1993). It was also interesting to note that age significantly co-varied with systolic (but not diastolic) blood

pressure yet did not co-vary with cortisol measurement. It is generally assumed that cortisol in response to challenge varies as a function of age (Otte et al., 2005). However, this study confirms previous findings by Lupien et al. (1996) that an age related change is not always observed. It is possible, however, that this sample of middle-aged volunteers will not reflect age related changes, since they do not include the 'elderly' and the age range of the sample was not wide enough to demonstrate a correlation.

In addition to physiological correlates of stress, various measures were administered to determine perceived psychological response to stress. These largely confirmed previous findings with respect to physiological and biological measures. This is consistent with the observation that females subjectively experience more stress than males, reporting more physical and psychological symptoms and reflecting a greater vulnerability (Bebbington, 1996; Kessler et al., 1981; Kessler and McLeod, 1984; Kroenke and Spitzer, 1998; Miller and Kirsch, 1987; Troisi, 2001). If this observation is combined with previous findings relating to cortisol responsivity, it could be argued that females respond psychologically to the stressor but not physiologically. This could lend further support to the view that females may be protected against the physiological effects of stress (Galea et al., 1997; Wolf et al., 2001).

#### **6.5.2.2 Efficacy of the TSST**

Assessing changes in salivary cortisol concentration across the session confirmed that greater responses occurred during the stress condition compared with the no stress condition. Baseline cortisol did not differ between the groups indicating that cortisol level was comparable prior to the experimental manipulation and did not differ between conditions. The TSST was successful in eliciting a greater cortisol response post task (evidenced by a percentage rise) with less recovery than in the no stress condition. These findings verify the use of the TSST and its ability to successfully induce stress and elevate salivary cortisol.

In the current study, the mean increase in cortisol was 26% in the stress condition. Schommer et al. (2003) found the TSST capable of eliciting a 2- to 4- fold increase in cortisol above baseline in just under 80% of subjects. Compared with Schommer, the elevation in cortisol observed, although significant by difference between conditions, was moderate as demonstrated in previous applications of this procedure. Kirschbaum



et al. (1993) reported that most volunteers displayed a minimum of a 2.5mmol/l increase as a result of exposure, which is consistent with the current study. Each volunteer exposed to the TSST displayed an elevation in cortisol and hence demonstrated a stress response. Concurrent with an observed physiological response, the stress condition was perceived to be significantly more stressful compared with the no stress condition as reported on the post session debrief questionnaire. Although no differences were observed in the ease of preparing for the tasks, preparation in the stress condition was perceived to be less successful than in the no stress task. Taken together, the findings confirm that the stress induction was successful in inducing a stress response that was both physiological and psychological prior to the cognitive testing period. This confirms that feasible comparisons in terms of responsivity were made and an accurate assessment of the influence of stress on cognitive performance.

### **6.5.3 Cognitive Performance by Condition & Stress Responses**

One of the main aims of this thesis was to explore the impact of stress-induced cortisol on cognitive performance in the centrally obese, who were hypothesised to demonstrate a greater response to the TSST and be vulnerable to stress induced cognitive impairment. Cortisol responses to stress were hypothesised to specifically impact on cognitive tasks that required hippocampal processing, for example, declarative memory tasks such as delayed recall on the Auditory Verbal Learning Task and paired associates learning task (PAL) in CANTAB. Of additional interest was the impact of cortisol on spatial tasks (SRM and SWM in CANTAB), which have been linked to hippocampal functioning. It was also of interest to explore subsequent cognitive performance in relation to gender and the possible interaction of gender and waist-hip ratio, since these have been shown to differ in response to stress responsivity and the cortisol profile.

#### **i. Auditory Verbal Learning Task**

Performance on the AVLT in both conditions demonstrated a similar learning curve i.e. a gradual increase in the number of words acquired from List A over trials one to five. Fewer words were acquired at each trial following exposure to the stress task compared with the no stress task. Similarly, the total number of words recalled (immediate verbal recall) was also less in the stress condition compared with the no stress condition. Finally, stress induction reduced the number of words recalled after a twenty-minute delay post learning (delayed verbal recall). These findings are consistent with previous

research confirming that stress induction has a detrimental effect on new learning and recall (Lupien et al., 1997). When performance was assessed in terms of actual cortisol and/or cortisol and blood pressure response (responders versus non-responders), stress was observed to reduce the number of words learned across the five trials. However, the effect of stress (cortisol and/or cortisol and blood pressure) on delayed recall was removed. This suggests that the cortisol response was insufficient or is not directly linked to delayed recall performance. It is possible that the acute response elicited by the TSST was insufficient to induce impairment. Wolkowitz et al. (1990; 1993) found that only high doses of exogenously administered glucocorticoids were sufficient to produce impairment, with no deficit observed at lower concentrations. Further, acute exposure may fail to induce impairment compared with longer-term exposure, even with lower concentrations of exogenous glucocorticoids (Newcomer et al. 1994). The greatest impairment is observed in elderly individuals who have experienced high basal levels of glucocorticoids over a considerable period of time, in addition, to heightened stress responses (Lupien et al. (1994; 1995). It was hypothesised that high waist-hip ratio individuals would exhibit altered basal cortisol (as in elderly individuals) with heightened stress responses that impact on cognitive performance compared with low waist-hip ratio individuals (whose cortisol profile both basally and in response to stress should be normal). However, the findings did not conclusively fit this hypothesis. High waist-hip ratio males in the stress condition recalled fewer words (total A) during trials one to five. This is consistent with a potentially elevated response to the stressor task but this response was not significantly greater than low waist-hip ratio males. It can be tentatively suggested that basal cortisol is disrupted in high waist-hip ratio males and so it is possible that the stress responses observed during the test session were insufficient to produce significant impairment. Hence, any impairment observed might be more related to disruptions in basal cortisol. This is difficult to ascertain, as this possibility has not been explored in previous literature. Furthermore, the observation that delayed recall was worse in high waist-hip ratio individuals irrespective of condition or cortisol response this suggests that impairments may be due to associated metabolic factors, for example, high blood pressure (Singh-Manoux and Marmot, 2005) and is consistent with elevated blood pressure responses to the stressor task. Waldstein and Katzel, (2005) specifically explored stress-induced blood pressure responses and observed diminished performance on immediate and delayed verbal memory. Messier et al. (2003) found that poor glucoregulation has been shown to impair performance on declarative memory

tasks and this was more evident in high waist-hip ratio individuals in the current study. Cortisol is also involved in the regulation of glucose, however, it is difficult to distinguish in the current study between precipitating factors.

## **ii. Paired Associates Learning (PAL)**

Like the AVLT, the paired associates learning task is an additional test of declarative memory. Stress exposure appeared to reduce performance in high waist-hip ratio individuals compared with low waist-hip ratio individuals but did not do so significantly. This is consistent with observed performance on the AVLT. However, unlike previous findings, performance in the no stress condition was significantly better in the high waist-hip ratio group, compared with low waist-hip ratio individuals. Moreover, stress appeared to improve performance in the low waist-hip ratio group. Stress has been shown to impair performance on this type of task (Young et al., 1999) while further facilitation of performance on various hippocampal tasks has also been previously reported in younger adults (Domes et al., 2002). A possible explanation for this observed difference in performance relates to the inverted 'U' hypothesis of cortisol receptor activity (Luine et al., 1993). Low waist-hip ratio individuals exhibit a lower mean basal cortisol secretion and minimal receptor occupancy, which neither impairs performance nor greatly improves it. Inducing a stress response and hence, elevating cortisol activity increases receptor occupancy to a point where performance is facilitated and could explain why performance might be improved in the stress condition. In the high waist-hip ratio condition, due to a potentially a higher basal cortisol activity (observed salivary cortisol levels were lower in females indicating a higher overall cortisol functioning) performance is already at or near optimum level. Inducing stress effectively tips the balance and pushes toward receptor over-occupancy, which could result in a decline in performance. However, impairment was observed in the analysis based on combined cortisol and blood pressure response to the stressor. This means that it is difficult to infer that performance is directly related to cortisol activity and not to changes in blood pressure. Blood pressure has been documented in previous research as a potential mediator of cognitive decline coinciding with central obesity (as the two co-occur). For example, Singh-Manoux and Marmot (2005) found that cognitive impairment was related to elevations in blood pressure in a sample of middle-aged men and women as part of the Whitehall II study and further confirmed by findings of Waldstein and Katzel (2005) who found diminished performance on immediate and

delayed verbal memory in relation to stress-induced blood pressure responses. Similar findings in older adults have also been reported by the Framingham study (e.g. Elias et al., 1993; 1995; 1997). Other factors such as insulin resistance and the incidence of diabetes have also been found to be inversely related to cognitive function (Convit, 2005; Elias et al., 2005; Hashizume et al., 2006). Indeed, insulin and glucose levels were found to be higher in those exhibiting a high waist-hip ratio in the current study and blood pressure responses mirrored the response pattern of cortisol, being significantly different by stress/no stress condition and in those with a high waist-hip ratio. To be able to attribute cognitive impairment in the centrally obese directly to cortisol requires further research in a larger sample with a wider range of waist-hip ratio.

### **iii. Spatial Recognition Memory**

Performance on the spatial recognition task was not directly related to stress/ no stress condition or to response group but to a combination of response group with waist-hip ratio and gender. A strong gender difference was observed with males performing significantly better than females on the task. In general, high waist-hip ratio males demonstrated poorer performance than low waist-hip ratio males in the stress condition. Impairment on this task was also evident when the combined cortisol and blood pressure response was used to indicate stress responsivity.

These findings are consistent with previous research that supports hippocampal involvement in visual spatial tasks (Lupien et al., 1999b; Reilly, 2000; Young et al., 1999). Further, it has been frequently documented that males out perform females on spatial tasks and that this is mediated by testosterone (Kimura, 2000; Voyer et al., 1995). Females perform better on tasks of verbal memory mediated by oestrogen, which has been demonstrated to preserve synapses (Le Blanc et al., 2001; Sherwin, 1997). In this research, this is supported by performance on the AVLT. However, the observation that performance in high waist-hip ratio males is poorer contradicts this idea and suggests that stress or central obesity may have modulated the influence of testosterone. The observation that deficits are further identified when actual cortisol responses are considered. This contributes to a more convincing argument for the adverse effect of stress-induced cortisol on performance for the spatial recognition task and further still in the centrally obese. Particularly, in males who previously showed better performance.

To conclude that the differences in performance are purely due to a gender difference requires further assessment.

In summary, small effects of stress per se were identified on a small range of cognitive tasks. Waist-hip ratio appeared to induce subtle alterations in performance but only on the AVLT and spatial recognition tasks. All other tests from the CANTAB battery failed to demonstrate any significant detriment in terms of stress, central obesity or gender. It is clear that cortisol alone does not mediate cognitive performance in this sample. Other metabolic factors need to be explored further in conjunction with stress responsivity. However, these findings suggest that subtle associations may exist.

#### **6.5.4 Basal Cortisol Profiles in High/Low WHR Males/Females**

##### **6.5.4.1 Basal Cortisol**

In an attempt to replicate the findings from Chapter Five, measures of salivary cortisol were obtained over the course of one day prior to the test session.

There was an observed differentiation between cortisol concentrations at waking and cortisol during the diurnal period. This indicated that the awakening response was distinct from the cortisol secreted during the remainder of the profile. It has been suggested that cortisol secreted in response to waking is under separate control to that secreted outside of this response and does not exclusively involve action of the hypothalamic pituitary adrenal axis (Born et al., 1999; Fehm et al., 1984; Spath-Schwalbe et al., 1991; Thorn et al., 2004).

Mean cortisol secretion across the profile, (incorporating both the awakening response and diurnal cortisol) was lower in the high waist-hip ratio group than the low waist-hip ratio group. However, no differences were identified using the various cortisol indices. The observation that cortisol secretion was reduced overall in the centrally obese is consistent with previous research findings (Ljung et al., 1996; Marin et al., 1992; Strain et al., 1980). Subjective perceived stress (measured prior to the study) was also significantly greater in high waist-hip ratio females, probably reflecting an underlying vulnerability or presence of HPA dysregulation. Mean cortisol concentrations across the awakening response and the diurnal period should be treated with caution and may not necessarily reflect a potential underlying difference. It is possible that the dynamic

change in activity during the awakening response altered the mean cortisol of the profile. Further, these observations were based on a single day of salivary cortisol assessment and hence may not be truly representative of each individual's usual profile. However, the differences observed in the current study replicated the findings from Chapter Five demonstrating some consistency in the findings.

#### **6.5.4.2 Metabolic Parameters & the Basal Diurnal Profile**

In order to further assess the relationship between cortisol and the collective symptomology of the metabolic syndrome (including central obesity) a range of blood biomarkers were assessed. Of these markers, glucose, insulin, triglycerides, IL-6, CRP and the degree of insulin resistance (using HOMA) were higher in those with central obesity who also showed lower levels of HDL. This confirms previous research findings (Bjorntorp and Rosmond, 2000; Hartz et al., 1984) and is compatible with the suggestion that those exhibiting a high waist-hip ratio also have a higher prevalence of diabetes and hypertension compared to those who exhibit a low waist-hip ratio.

To assess the hypothesis that cortisol is linked to the incidence of the metabolic syndrome, the correlation of cortisol with metabolic syndrome symptomology was explored. Basal cortisol co-varied with glucose of the biomarkers assessed. However, cortisol regulates glucose activity as part of gluconeogenesis (Miller and Tyrell 1995) which supports this finding but provides little support for the view of metabolic syndrome as a neuroendocrine disorder. However, further analysis provided more substantial support for the hypothesis that the metabolic syndrome is a neuroendocrine disorder, characterised by an underlying HPA dysregulation with overt cortisol disruption (Bjorntorp and Rosmond, 2000; Rosmond et al., 1998; 1999b). A greater incidence of metabolic symptomology (namely elevated glucose, insulin, level of insulin resistance, and the inflammatory marker IL-6) co-occurred with non-classic diurnal cortisol profiles. A non-classic profile may signal vulnerability to HPA dysregulation and hence 'pathological' cortisol secretion. These findings replicate those described in Chapter Five and suggest that disrupted basal cortisol secretion may contribute to the development of the metabolic syndrome by increasing vulnerability (Bjorntorp and Rosmond, 2000). Replicating the findings from Chapter Five, lends weight to this hypothesis. However, further research is necessary to fully ascertain the role of cortisol in metabolic syndrome, since volunteers in the current study did not

have the syndrome nor was the research design adequate to determine the likelihood that many might develop it. As pathological cortisol secretion can occur over many years of chronic stress exposure, it may be that the occurrence of the metabolic syndrome is a gradual process. Therefore, assessment of cortisol activity alongside metabolic syndrome symptomology in a longitudinal study may be more appropriate.

#### **6.5.5 Methodological Issues & Study Limitations**

There are, as with any piece of research, methodological issues and study limitations that should be acknowledged when interpreting the research findings. These will be discussed in the following section.

As in Chapter Five, the issue of compliance and the exclusion of individuals based on poor profiles should be considered. The findings in the current study replicated Chapter Five in that 'non-classic' diurnal profiles, that might otherwise have been removed from the dataset were shown to be associated with an increased incidence of subjective stress reporting and metabolic syndrome symptomology. The prevalence of a classic profile in the current sample was only 53%, which is very similar to the 52% reported in Chapter Five. This underlies the need to consider the exclusion process in studies of this nature to prevent key populations of interest being excluded from analyses, which might have had the effect of reducing the likelihood of finding effects.

High waist-hip ratio females were found to be the least cortisol responsive subgroup in the study. This contradicts previous research findings, which suggest that females with central obesity are more responsive to stress. Primarily, findings from Epel et al. (2000) demonstrated a pronounced cortisol response to stress in high waist-hip ratio females. This has been confirmed by previous studies (Uhart et al., 2006) and attributed to a decrease in HPA feedback sensitivity (Born et al., 1995; Heuser et al., 1994; Gallucci et al., 1993). This was not observed in the current study. It is possible that the discrepancies highlighted in terms of the lack of responsivity in females and high waist-hip ratio females in particular may be due to methodological issues. Epel et al. (2000) implemented a series of stressors to measure cortisol responsivity and habituation. In the current study, a single episode of stress exposure was employed, which may have been insufficient to induce the level of responsivity observed by Epel in high waist-hip ratio females. Furthermore, comparisons were made amongst a small number of

volunteers when comparing within condition, gender and waist-hip ratio groups. The sample size in the high waist-hip ratio male group is, in particular, small in comparison to other groups. The groups relied on volunteers and may not be truly representative. It is also possible that this small sample size could have inflated effects or lead to a failure to detect effects (type II error).

An important issue to consider in a study of this nature is volunteer compliance, an issue that is often reported in this type of research (Kudielka et al., 2003) as previously discussed. This relates specifically to saliva collections for cortisol assessment. The majority of the samples for basal cortisol assessment are collected by volunteers at home or at work away from the research unit. This issue is identical to that encountered in Chapter 5 (See Section 5.5.4) and concerns the accuracy of the timing of each sample and the volume of saliva obtained. The issue of compliance was in part removed during the experimental session, as the researcher was present to prompt each sample and to try to ensure adequate volumes. During a procedure such as the Trier Social Stress Test this is particularly difficult as inducing a stress response by public speaking frequently induces a dry mouth, which makes saliva collection difficult. This is a consequence of the stress induction procedure that is ultimately unavoidable. If individuals are responsive to the procedure, they are more likely to experience a dry mouth. This could mean that those individuals who show the greatest responsivity might be those who are most likely to be excluded for failing to provide a saliva sample of adequate volume. Hence, this is something that must be taken into consideration when designing studies of this nature. It is of paramount importance that the TSST is conducted in a professional manner in order for it to have the most impact. Greater adherence to the protocol results in a greater stress response. It is commonly observed that responses are not as elevated as in a truly stressful situation due to the essentially unrealistic nature of the procedure. As with all experimental procedures, care must be taken when generalising the findings. It is also of interest to consider the range of volunteers within the sample, with consideration for each individual's background. For example, those in the profession where public speaking is a regular task may respond differently to the TSST than those who rarely if ever undertake such a task, i.e. prior experience of the various aspects of the task may lead some individuals to develop 'immunity' to it.

In the current study, it would have perhaps been more efficacious to employ a repeated measure design in order to assess individual performance on the tasks under both



condition of stress and condition of no stress and to reduce between group variation. However, the nature of the TSST, this means that there is a great potential effect or interference of anticipation on subsequent exposure to the task. The lack of baseline cognitive data is a major weakness in the current study and limits the reliability of the findings. An alternative approach, would have included assessment of cognitive performance prior to the onset of the stress/no stress tasks. However, there are three issues with this approach. The first concerns the length of the experimental session. In the current study, the session duration lasted two hours with approximately one hour of cognitive testing. Any increase on this is certain to induce fatigue effects and would reduce the accuracy of the results and add to volunteer discomfort. Second, pre and post cognitive testing would increase the likelihood of practice effects on the cognitive testing even if counterbalancing of the tasks was employed. Finally, the stressfulness of the cognitive battery itself must be ascertained, as there is the potential for the cognitive testing to induce a stress response. Bohnen et al. (1990) specifically employed a cognitive test battery as an effective stress induction tool; similarly, Lupien et al. (1997) found a crossover of response when basal measures of cognitive performance were obtained prior to the stress induction demonstrated by an early cortisol response that could have been attributed to the test battery. This demonstrates the potential for the procedure to create substantial noise in the data. To try to address the lack of baseline cognitive performance data in the current study, IQ was estimated from the National Adult Reading Test and used as a covariate to provide adjustment for IQ if necessary. Further, due to the characteristics of the sample assessed (waist-hip ratio, gender, condition etc) only a small number of volunteers were included per cell in some of the analyses with a consequent reduction in the power to detect effects. Therefore, any generalisations from the current data set must be treated with caution. It is of necessity that these findings are to be replicated in a much larger sample to increase the reliability of the findings. When these issues are considered, there is scope for improvement in the reliability and validity of the findings obtained.

## **6.6 Conclusions**

The main findings of this study suggest that males are more responsive to stress than females, and specifically high waist-hip ratio males may secrete more cortisol during stress exposure. Performance in high waist-hip ratio males was poorer on some tasks of declarative memory indicating that the hypothesis for increased vulnerability to

impairment in this subgroup may be supported. However, impairments on some tasks were only maintained when blood pressure responses were considered indicating that other physiological and metabolic parameters should be considered in this relationship. In a replication of findings in Chapter Five, classic and non-classic diurnal profiles differed in relation to the expression of metabolic syndrome symptomology and psychological characteristics such as perceived stress. This provides further support for the role of altered cortisol secretion in the development of metabolic syndrome. The study also raised important issues relating to data screening and exclusion procedures.

In conclusion, the findings of the study presented in this chapter suggest that a subtle association between cortisol and central obesity may exist. Other metabolic factors are involved in the determination of stress responsivity and in the manipulation of cognitive performance. Exploring the direct influence of central obesity through action of cortisol is novel and these findings are encouraging for future research to further explore the mechanisms involved.

## CHAPTER SEVEN

### General Discussion

---

---

This final chapter is a general discussion of the main research findings of this thesis in relation to the original aims discussed in Chapter Two. The chapter will explore the implications of these findings for current research and what future research is suggested as a result of this thesis. The methodological limitations of the thesis will be discussed.

#### 7.1 General Aims

This thesis represents a detailed exploration of stress responsivity in individuals with central obesity marked by the activity of the glucocorticoid, cortisol. Salivary cortisol activity, both basal and stimulated in response to stress was explored in those who exhibit central obesity (high waist-hip ratio) and those who do not (low waist-hip ratio). Further, the potential for altered cortisol response to stress to influence cognitive performance was assessed.

#### 7.2 Basal Cortisol in High and Low Waist-Hip Ratio Individuals

##### 7.2.1 Reproducibility and Consistency of the Cortisol Diurnal Profile

A pilot study of the use of saliva samples for basal salivary cortisol measurement in a sample of older adults formed the first study in this thesis. This was conducted to explore the natural circadian activity of cortisol in the form of a diurnal profile. As the literature review in Chapter One suggested, the diurnal profile of cortisol is a plot of basal salivary cortisol concentration against time post waking and incorporates the cortisol awakening response, a dramatic change in cortisol activity during the first 30-45minutes post waking (Prüssner et al., 1997). The profiles, once constructed, were compared using a number of cortisol indices (Edwards et al., 2001; Wüst et al., 2000a; Schmidt-Reinwald et al., 1999) described in Chapter Three (Section 3.3.3). These are computational calculations based on the shape of the cortisol profile obtained and the mean level of cortisol activity during a sampled day. The practicalities and

methodological issues of assessing cortisol in this way were explored by this pilot study.

The findings suggested that a substantial degree of inter-and intra-subject variability exists in the basal cortisol diurnal profile which needs consideration when exploring an individual's 'typical' basal cortisol activity. The consistency of the profile should be determined prior to aggregation to preserve the accuracy of the final aggregate profile that is analysed in relation to other outcome variables. The findings also suggested that more than one day of sampling is necessary to accurately reflect underlying basal activity and that sampling over 3 days in the current study provided an more informative insight into variations in basal cortisol activity. It was observed that the prevalence of a significant cortisol awakening response and 'textbook' profile were low, even in a small sample. It was apparent that variations were due to more than just an issue of compliance and this was the subject of further investigation in the subsequent studies presented in this thesis.

The observation that substantial variation in the basal profile was obtained conflicts with previous research findings which suggested that moderate consistency is usually observed when measuring cortisol in this way (Wüst et al., 2000). However, it was observed that, there was a tendency for some consistency in the nature of the profile. For example, if an individual exhibited a poor profile on one of the sampling days, then the likelihood that a poor profile would be exhibited on all three of the sample days was high. Similarly, those exhibiting a profile with an identifiable diurnal profile shape, tended to exhibit a similar profile across the three days with some variation within that profile.

Above all, this study was important in exploring some of the methods used in basal saliva cortisol measurement that were to be used in the subsequent studies of this thesis. In terms of the data obtained, it was of great importance is to realise that only tentative claims can be made from a small sample size and that the points raised required further assessment. This was explored in more detail in Chapter Five.

### **7.2.2 Patterns of Basal Diurnal Cortisol in High and Low Waist-Hip Ratio Individuals in Relation to Psychological and Metabolic Parameters.**

After exploring the feasibility and practicality of the use of saliva samples for cortisol measurement, the basal activity of cortisol in the centrally obese was examined. Previous research suggests that cortisol production in the centrally obese is elevated, however, this elevation is not readily detectable in the basal circadian profile. This is due to an enhanced cortisol clearance rate (Ljung et al., 1996; Marin et al., 1992; Strain et al., 1980). Salivary cortisol measurements taken over the diurnal period in centrally obese individuals are suggested to be lower in concentration than in non-centrally obese volunteers (Duclos et al., 1999). As a result of cortisol hypersecretion, diurnal profiles have demonstrated a blunted cortisol awakening response with comparable diurnal cortisol concentrations (Akana, et al., 1992; Dallman et al., 1993).

The prevalence of a classic diurnal profile within the sample, examined in Chapter Five, was assessed, with a view to identifying non-classic diurnal profiles in those with central obesity. This was based on the observation that the diurnal profile of cortisol differs with the incidence of central obesity (Andrew et al., 1998; Ljung et al., 2000; Phillips et al., 1998; Phillips et al., 2000; Rask et al., 2002; Van Cauter et al., 1996; Wallerius et al., 2004; Ward et al., 2003). However, the accuracy and reliability of the profiles obtained is often confounded by the issue of volunteer compliance (Kudielka et al., 2003). Deviations from a textbook or 'classic' profile are presumed to be due to non-compliance but may be more related to actual metabolic and psychological variables of interest (Rivera and Svec, 1989). The psychological and biological characteristics of 'non-classic' profiles were assessed to explore what factors were associated with 'non-classic' profile expression. Furthermore, the observation that central obesity and cortisol dysregulation co-occur, for example in patients with Cushing's syndrome (Starkman et al., 1999; 2003), suggested that cortisol is implicated in the expression of the metabolic syndrome. Rosmond et al. (1998; 1999b) assessed salivary cortisol in relation to central obesity and demonstrated pathological cortisol secretion in males with metabolic syndrome symptoms. The metabolic syndrome has been labelled a neuroendocrine disorder of cortisol dysregulation (Bjorntorp and Rosmond 1999; 2000; 2001). Therefore the relationship between cortisol activity and metabolic symptoms was assessed. Further, the incidence of metabolic symptomatology in those who exhibit a non-classic profile was explored.

The findings suggested that profiles in high waist-hip ratio individuals were almost identical to those exhibited by low waist-hip ratio individuals with no difference in shape. However, the mean level of cortisol secreted across the diurnal profile did differ between high and low waist-hip ratio individuals. High waist-hip ratio individuals exhibited significantly lower cortisol across the diurnal profile compared with low waist-hip ratio individuals. No differences in the cortisol awakening response were observed with exception that high waist-hip ratio individuals secreted less mean cortisol in response to waking than low waist-hip ratio individuals. Further, high waist-hip ratio females exhibited the smallest cortisol awakening response out of the four groups. These findings were replicated in Chapter Six. A high prevalence of non-classic profiles was observed in the current study relative to the classic 'textbook' profiles. Profiles that deviated from a 'classic' profile ('non-classic') were associated with a significantly greater vulnerability to insulin resistance, higher levels of triglycerides and a significantly greater concentration of inflammatory markers, specifically c-reactive protein. Although not significant, non-classic profiles were associated with higher cholesterol, IL-6, LDL and lower HDL levels. Non-classic diurnal profiles were also associated with an increased incidence of subjective reporting of the intensity of daily hassles and poorer subjective sleep quality.

#### **(i) The Basal Cortisol Profile and Central Obesity**

The observed difference in the mean level of cortisol between high and low waist-hip ratio individuals is consistent with previous research findings. Individuals with central obesity exhibit elevated cortisol levels in a state reminiscent of Cushing's disease (Bjorntorp and Rosmond, 2000). However, measured basal cortisol concentrations in saliva are observed as being lower in the centrally obese than in lean or peripherally obese individuals as demonstrated in the current findings. This has been observed in both morning salivary cortisol (Ljung et al., 1996; Marin et al., 1992) and diurnal cortisol concentrations (Duclos et al., 1999). It has been proposed that individuals with central obesity are capable of enhanced breakdown of excess cortisol evidenced by an increased urinary output of cortisol metabolites (Lottenberg et al., 1998). In light of this observation, the current findings offer only partial support for altered cortisol secretion in the centrally obese. As no measure of urinary output was made, it cannot be clearly determined whether cortisol hypersecretion is occurring as the current study provides no evidence of an enhanced clearance capability since urinary metabolites were not

measured. Despite the consistency of the current findings with previous research, further research is required to explore the possibility that HPA dysregulation underlies potential cortisol hypersecretion in those with central obesity.

### **(ii) The Basal Cortisol Profile and Sleep Quality**

The observation that non-classic profiles were associated with significantly greater reporting of daily hassles (intensity of) and poorer sleep quality is also consistent with previous findings (Ockenfels, 1995; Stone et al., 2001; Backhaus et al., 2004). More specifically, the relationship between sleep quality and cortisol secreted during the cortisol awakening response may offer support to the role of the HPA axis in the control of the cortisol awakening response, or at the very least, suggests that the HPA axis is capable of substantial influence. The debate as to whether the cortisol awakening response is under the control of the HPA axis or the Suprachiasmatic nucleus (SCN) in the hypothalamus (Chapter One, section 1.3.2, part ii) is longstanding. The cortisol awakening response is affected by changes in sleep patterns that collectively produce spontaneous waking (Born et al., 1999; Spath-Schwalbe et al., 1992). The findings of the current study suggest that it cannot solely be the SCN which is in control of the cortisol awakening response. The finding that altered cortisol profiles (non-classic) are associated with poorer sleep quality and a greater intensity of daily hassles suggests that stress (and the HPA axis) is still very much in control. To fully determine the weighting of this influence requires further research.

### **(iii) The Basal Cortisol Profile and Metabolic Syndrome**

The collective increase in metabolic symptomatology in those who exhibited a 'non-classic' diurnal profile observed in this study provides some evidence that cortisol may be linked in some way to the metabolic syndrome and is consistent with previous research. The proposed relationship between cortisol and the metabolic syndrome was initially based on the observation that insulin resistance, the main symptom of the metabolic syndrome, is linked to cortisol activity. Cortisol has a primary role in the process of gluconeogenesis (Miller and Tyrell 1995) and therefore may contribute to the expression of impaired glucose tolerance and insulin resistance, particularly in the centrally obese. This linkage led to the suggestion that cortisol could directly underlie the expression of the syndrome and the proposal that metabolic syndrome is, in essence, a neuroendocrine disorder (Bjorntorp and Rosmond, 2000). Bjorntorp and Rosmond,

(2000) suggested that flattened profiles with lower morning cortisol or 'pathological' cortisol secretion were associated with metabolic symptomology, endocrine abnormalities and central obesity. Further, Rosmond et al. (1998; 1999b) observed pathological basal cortisol secretion in males who exhibited symptoms of the metabolic syndrome, from assessment of basal cortisol profiles. The findings of this thesis provide some support for the proposed relationship between cortisol and each individual biomarker of the metabolic syndrome. The findings of Rosmond et al. (1998; 1999b) were based on a statistical weighting procedure applied to cortisol data within the basal profile of which was not applied to the current data set. Consequently, using this statistical approach may provide further support for the role of cortisol in the metabolic syndrome. To fully examine this relationship may be beyond the scope of this thesis and not possible with the modest sample in each study. However, in light of the observation that cortisol dysregulation should precede the metabolic syndrome, the finding do suggest a relationship. Further, research to explore this in more detail is essential.

#### **(iv) Cortisol, Central Obesity and Inflammation**

An alternative hypothesis for the relationship between cortisol activity and central obesity is based on the observation that chronic stress exposure produces a state of chronic inflammation, which is associated with central obesity and metabolic syndrome symptomology (Black, 2006). This process is more mediated by the stress hormones epinephrine, norepinephrine and cortisol, which collectively promote fat accumulation and promote an inflammatory state marked in the visceral tissue of central obesity. This contributes to the development of insulin resistance and the metabolic syndrome and presents as a risk for cardiovascular disease and type II diabetes. Black (2006) argues that the linkage of fat accumulation and an inflammatory state is further supported by the observation that weight loss reduces the concentration of inflammatory markers present. Further, the neural pathways linking the brain to fat deposition are the same as those mediating stress responses. The hypothesis that central obesity and associated metabolic syndrome symptomology are an inflammatory response resulting from chronic stress exposure is supported by the current findings since the inflammatory markers IL-6 and CRP were elevated in those with central obesity. CRP also demonstrated significant covariance with mean measures of cortisol sampled during the same period. To ascertain fully, the feasibility of this theory, further research is required



including assessment of a much wider range of inflammatory markers and change in these in relation to weight loss.

Building on the idea that the relationship between stress and obesity is mediated through inflammation, the relationship between stress and immune status must be considered. Psychological stress is known to affect immune status leading to a vulnerability to infectious disease (Herbert and Cohen, 1993). As discussed in Chapter One (section 1.3.2, part iii), elevations in cortisol from chronic stress could mediate the change of night time Type 1 activity to daytime Type 2 activity (Edwards et al., 2003; Petrovsky and Harrison, 1995; 1997). Previous research has observed that abnormal cortisol profiles have been associated with certain pathologies including cancer and AIDS (Sapsee, 1997). Further, flattened profiles have been found to be predictive of earlier mortality particularly in breast cancer patients (Sephton et al., 2000). There is some evidence to suggest that stress reactivity is associated with a suppression of the immune system (Marsland et al., 1995). It is possible that difference in endocrine and cardiovascular activity (reactivity) may regulate immune responses, increasing vulnerability to infection and disease, and that this might be greater in those exhibiting central obesity. Indeed, advanced disease states such as Type II diabetes have been suggested to be an immune system disorder (Pickup and Crook (1998). This is because in type II diabetes, there is a cytokine associated acute phase response, consistent with the observation that stress can induce an acute phase response and which is associated with the onset of metabolic diseases (Black, 2003). This suggests that collectively chronic stress (in the form of elevated cortisol) via interactions with metabolic factors and the immune system promotes a vulnerability to a poorer prognosis for health.

### **7.3 Cortisol Responses to Stress in High and Low Waist-Hip Ratio Individuals**

Once basal cortisol activity in those with central obesity had been established, an assessment of stress responsivity was made. Despite the absence of pathological basal salivary cortisol secretion in those with central obesity, it was postulated that further evidence for a dysregulated HPA axis would manifest in the form of an exaggerated response to stress. Cortisol responses to a psychological stressor are elevated in the centrally obese (Epel et al., 2000; Marin et al., 1992; Moyer et al., 1994) compared with lean (Moyer et al., 1994; Marin et al., 1992), peripherally obese and non-obese individuals (Epel et al., 2000).

Analysis of the cortisol response to the stressor suggested that high waist-hip ratio males secreted the largest cortisol response to the stressor. However, because high waist-hip ratio males exhibited greater cortisol at baseline, prior to stress exposure, high waist-hip ratio males did not demonstrate a greater response. As a result, low waist-hip ratio males appeared to exhibit the largest response. The observed elevation in cortisol at baseline conflicted with previous findings, which suggested that basal cortisol is reduced in those with central obesity. This led to the possibility that high waist-hip ratio males were demonstrating an anticipatory response. In a separate analysis using a comparable sample time point from the prior Monitoring Day One as an alternative baseline in the response calculation, high waist-hip ratio males were found to be the most responsive in comparison to the remaining members of the sample (Appendix 22). Blood pressure response to stress was greater in males and further in high waist-hip ratio males compared with low waist-hip ratio males. Less recovery was observed in the high waist-hip ratio males compared with low waist-hip ratio males who demonstrated good HPA regulation (evidenced by cortisol changes post task). High waist-hip ratio females demonstrated the smallest change in cortisol in response to the stressor task.

The observation that those with central obesity secrete more in response to a stressor than lean or peripherally obese individuals is consistent with previous research (Epel et al., 2000; Marin et al., 1992; Moyer et al., 1994). However, such studies reported that increased stress responsivity was apparent in females with central obesity but not males. The literature examining increased responsivity in centrally obese males is sparse. Further, the observation that in the current thesis, females were least responsive contradicts previous findings. Epel et al. (2000) observed that greater cortisol responses to a stressor were evident in females with central obesity compared with lean and peripherally obese females. It has been suggested that hyper-responsivity occurs as a consequence of impaired HPA regulation (Born et al., 1995; Heuser et al., 1994; Gallucci et al., 1993). The lack of stress responsiveness in females may be linked to sex hormones and their protective influence. Wolf et al. (2001) proposed that females are protected from over-responding to stress by the specific action of oestrogen (Galea et al., 1997). Previous studies have demonstrated a blunted response to stress after a period of oestrogen supplementation (Kudielka et al., 1999). This lack of response in females may concurrently cause males to appear more responsive when faced with a stressor (Wolf et al., 2002). In addition to the influence of oestrogen, oral contraceptive use can

influence stress responsivity. Kirschbaum et al. (1999) observed that salivary cortisol responses to the TSST were significantly lower in females taking oral contraceptives which contributed to an observed gender difference in the salivary cortisol response to stress. However, no differences were observed for total plasma cortisol or ACTH responses. Although, females currently using oral contraceptives were not excluded from participating in the research contained in this thesis, only 3 of 70 were taking oral contraceptives. This is unlikely to account for the lack of stress response in females.

Previous studies have demonstrated increased stress responsivity in males (Kirschbaum et al., 1995; Wolf et al., 2001) in both younger (Kirschbaum et al., 1992; 1995a, b) and older adults (Kudielka et al., 1998). The possibility that centrally obese males could demonstrate an enhanced response to stress due to sex hormones is little researched. Centrally obese males often show lower levels of testosterone (Derby et al., 2006). Further, testosterone has been shown to inhibit HPA activity, particularly the release of ACTH (reviewed by Williamson et al., 2005). Lower levels of testosterone in centrally obese males could signal increased responsiveness to stress, which would concur with the current findings. This postulation however, has not been assessed and so requires further research in studies which measure circulating (unbound) testosterone.

In summary, a gender difference in the response to stress between males and females was observed. The likelihood that this difference was due to central obesity is small and is more likely due to the influence of sex hormones in a gender mediated response. Sex hormones should be measured in a study of this nature, to fully ascertain this hypothesis.

#### **7.4 The Effect of Psychological Stress on Cognitive Performance in High and Low Waist-Hip Ratio Individuals**

It has been established previously that stress induced elevations in cortisol result in cognitive impairment on hippocampal tasks, particularly declarative memory (Lupien et al., 2005). Cognitive decrements have been observed in the centrally obese who also show insulin resistance, high blood pressure and other features of the metabolic syndrome (Convit, 2005; Elias et al., 2005; Hashizume et al., 2006). However, little research has specifically explored the impact of stress induced cortisol in the centrally obese on cognitive performance. Heightened stress responses in the centrally obese

imply greater cortisol and hence a greater vulnerability to cognitive impairment. This thesis is novel in the examination of this hypothesis.

The potential for stress induced cortisol to impair subsequent cognitive performance was explored. It was of particular interest to assess cognitive performance in those with central obesity who, it has been argued, may be more vulnerable to cognitive impairment. The results demonstrated significant impairment on tasks that are most clearly associated with hippocampal processing. Specifically, stress induction (TSST) impaired performance on the immediate verbal recall component (total number of words recalled) of the auditory verbal learning task (AVLT). Delayed recall was also reduced in those in the stress condition. The total number of words recalled across the initial five trials was lower in high waist-hip ratio males. This impairment was maintained when actual cortisol responses and combined cortisol and BP responses were considered. However, performance on the delayed recall component of the AVLT when cortisol and blood pressure responses were considered was not impaired. Performance on the paired associates learning task was also poorer in the stress condition; further so in high waist-hip ratio males within the stress condition. However, in the no stress condition, high waist-hip ratio males performed better than low waist-hip ratio males. Further, poorer performance was observed in high waist-hip ratio individuals who demonstrated both a cortisol and blood pressure response to the stressor task. Performance on the spatial recognition task was poorer in high waist-hip ratio individuals, in addition to an overall gender difference with males performing better than females. When actual cortisol responses were considered, high waist-hip ratio responders showed poorer performance. Further, when combined cortisol and blood pressure responses were considered, performance was again poorer in high waist-hip ratio male responders. High waist-hip ratio female responders also demonstrated poor performance. Unfortunately due to cell frequencies it was not possible to include condition and response in the same analysis. The study therefore requires replication with a large sample size across all cells.

The finding that performance was poorer for tasks that involved hippocampal processing after stress exposure is consistent with previous research findings (De Kloet et al., 1998; McEwen et al., 1995; 1997). This was true for comparisons between the stress and no stress conditions and further, in terms of actual cortisol responses (responders versus non-responders). It was also observed that high waist-hip ratio

individuals demonstrated poorer performance on certain tasks (AVLT, PAL, and SRM) as hypothesised. This provided some support for the idea that chronically elevated cortisol levels in those with central obesity may present a vulnerability to cognitive impairment after stress exposure. These findings however are not conclusive and require further investigation. At the time of writing, no previous assessment of cortisol induced cognitive impairment in those with central obesity has been made in the literature, hence there is no comparative research. Previous studies exploring cognitive deficits in those with central obesity have identified impairments in tasks of declarative memory as a result of poor glucoregulation (Messier et al., 2003). Other studies have identified impairment related to the incidence of impaired glucose tolerance (for a review, see Messier et al., 2005) and type II diabetes (Bent et al., 2000; Goldstein et al., 2001; Strachan et al., 1997). The absence of impairment on the delayed recall task when only cortisol responses are considered, suggests that it cannot be cortisol alone which is mediating the effect. This is further confirmed since the impairment is evident when a combined cortisol and blood pressure response is included as a between subjects factor. Previous research has demonstrated that high blood pressure and hypertension can cause impairment on certain cognitive tasks (Singh-Manoux and Marmot, 2005). It may be that additional metabolic factors are collectively involved in mediating impairment.

Performance on the paired associates learning task was also poorer in response to the stressor, which is again, consistent with previous research (Young et al., 1999). This was maintained when actual cortisol responses to the stressor were considered. Poorer performance was observed in high waist-hip ratio individuals in the stress condition as hypothesised and consistent with findings on the AVLT. However, interestingly in the no stress condition, performance was better in high waist-hip ratio individuals than in low waist-hip ratio individuals whose performance was worse. It is hypothesised that this pattern of impairment can be linked to the inverted 'U' hypothesis of glucocorticoid and mineralcorticoid receptor activation. In high waist-hip ratio individuals, basal levels of cortisol are postulated to be high and so receptor occupancy is at an optimal rate. Therefore under conditions of no stress performance is good. Elevating cortisol further via stress exposure pushes receptor occupancy to the limit and impairment occurs. At the other end of the scale, in low waist-hip ratio individuals, receptor occupancy is low due to low/normal basal cortisol and so performance is below optimal. In conditions of stress, receptor occupancy increases toward optimal levels and so performance

improves. That is not to say that everyday cognition is poor in low waist-hip ratio individuals, it would simply appear poorer in comparison to better performance in high waist-hip ratio individuals. At this stage, it is impossible to ascertain if this theory is feasible due to the lack of comparable research in this area. Yet it does coincide with the observation that hippocampal volumes in those with central obesity are often reduced (Jagust et al., 2006). This would be an anticipated consequence of chronic glucocorticoid exposure (Sapolsky et al., 1986).

Consistent with the AVLT and paired associates learning task, performance on the spatial recognition task was poorer as a result of stress exposure. Spatial memory is also mediated by the hippocampus and so these findings are consistent with previous research (Lupien et al., 1999b; Reilly, 2000; Young et al., 1999). The observation that high waist-hip ratio individuals demonstrate poorer performance is perhaps the most convincing evidence for the influence of cortisol on cognition in the centrally obese. It has been frequently documented that males perform better on tasks of spatial memory than females (Kimura, 2000; Voyer et al., 1995) and this was reflected in the findings. Yet high waist-hip ratio males demonstrated poor performance on this task. This suggests the possibility of a strong combined influence of cortisol and central obesity on cognition, especially as this impairment was maintained when actual cortisol responses were considered. As previously discussed, it has been observed that those with central obesity exhibit lower testosterone (Derby et al., 2006). Lower testosterone has been associated with poorer memory performance (Barrett-Connor et al., 1999; Moffat et al., 2002). Testosterone is also linked to HPA activity (Williamson et al., 2005). It may be that these factors collectively mediate subsequent impairment. Teasing out the relative contribution of these predictors of impairment requires further research, which is beyond the scope of this thesis.

In summary, the current findings offer some clear insight into the potential for psychological and metabolic factors to collectively mediate cognitive performance. The evidence for cortisol as a key factor is promising.

## **7.5 Methodological Issues and Limitations**

The studies contained in this thesis possess certain methodological weaknesses that limit the conclusions of which can be drawn from the research. These will be discussed in the following sub-sections.

### **7.5.1 The Assessment of Basal Cortisol in the Centrally Obese**

An assessment of basal cortisol in those with central obesity suggested that basal cortisol differs between high and low waist-hip ratio individuals. The findings demonstrated that basal salivary cortisol was lower in high waist-hip ratio individuals. This was consistent with the hypothesis that centrally obese individuals suffer elevated basal cortisol but demonstrate normal or less than normal concentrations in saliva due to an enhanced clearance capability. However, despite the consistency in findings, it cannot be fully ascertained using the current data set that an enhanced clearance capability exists. To explore fully this hypothesis, would require an assessment of urinary metabolites. An elevation in excreted cortisol metabolites in those with central obesity would offer support for an excess cortisol breakdown (Lottenberg et al., 1998) and would provide evidence for cortisol hypersecretion in those with central obesity. Further, an assessment of nocturnal cortisol responses to waking should be made. Previous research suggests that cortisol responses to nocturnal waking would result in a compensatory lowering of peak morning cortisol levels (Akana et al., 1992) so that the net mean cortisol during the 24-hour circadian rhythm does not change. This would further assess the ability of the HPA axis to regulate itself and would be a more comprehensive assessment of cortisol activity in those with central obesity.

### **7.5.2 Efficacy of the TSST**

Observed cortisol responses to the Trier Social Stress Test (TSST) suggested that the procedure was efficacious in stimulating a HPA response. Previous research has shown the found the TSST capable of eliciting a 2- to 4- fold increase in cortisol above baseline in 80% of subjects (Schommer et al., 2003). Further, Kirschbaum et al. (1993) reported that most volunteers displayed a minimum of a 2.5mmol/l increase as a result of exposure. Although each volunteer who was exposed to the stressor task demonstrated a cortisol response, not every increase was quite so profound. There was great variation in cortisol response, suggesting that although the TSST was essentially

effective as a stress induction tool, it may not have reached full potential in terms of stimulated cortisol response. The characteristics of the testing environment are important in inducing a stress response, for example, Kuhlman and Wolf (2005) demonstrated greater responsivity in a formal testing environment compared to a relaxed environment and further, that this lead to greater impairment in post stress cognitive assessment.

In terms of an observed gender difference in the response to the stressor, it may be possible that the qualities of the stress tool induced differing responses. The TSST comprises elements of social-evaluative threat and uncontrollability. It is possible that these elements may be appraised differently in males than females and hence results in differing cortisol responses manifesting as a gender difference. This may link with the observation that greater perceived stress can produce a greater cortisol response to the TSST (Fries et al., 2006). Should a different tool for stress induction be used, the same responses may or may not be observed. However, no gender differences have been noted in terms of the efficacy of the TSST (Dickerson and Kemeny, 2004) and it has been shown to be the most effective stress induction technique.

In the assessment of stress responsivity in those with central obesity, Epel et al. (2000) implemented a series of stressors to measure cortisol responsivity and habituation. In the current study, only a single episode of stress exposure was employed. This may offer a partial explanation for the lack of stimulated cortisol response in high waist-hip ratio females. A single exposure may have been insufficient to induce the level of responsiveness observed previously. However, this does not explain the opposing cortisol responses exhibited in high waist-hip ratio males. To assess this further, response to repeated stress should be assessed to more comprehensively assess stimulated cortisol responses in this sub-group.

### **7.5.3 Assessing Cognitive Performance**

Perhaps the most significant weakness in the assessment of cognitive performance is the lack of baseline cognitive data prior to exposure to the stressor task. This places the reliability of the cognitive assessment into question. Due to time constraints and the nature of the test session as it stands, additional assessment of baseline performance was deemed impractical. To compensate for this, an estimate of IQ (using the National Adult



Reading Test) was made and included as a covariate to control for IQ related differences in performance. This eliminated the possibility of practice effects often observed with the repeated use of cognitive test batteries and further the possibility of the battery inducing a significant anticipatory response prior to stress exposure or acting as a stressor per se, as observed in previous research (Lupien et al., 1997). Neuropsychological test batteries have been implemented as stressors in their own right (Bohnen et al., 1990). However, it cannot be fully ascertained whether poor performance post stressor was also poor prior to testing. Cognitive ability can differ by cognitive task, despite variance in IQ. An example of this is the difference in performance between males and females for spatial memory. Males generally perform better on spatial tasks than females. The method of encoding information can also affect retrieval. Therefore, to provide a more comprehensive assessment of declarative memory, it would be of interest to assess visual verbal memory, both recall and recognition. Further, in addition to proactive interference, retroactive interference should be assessed. This is the potential for newly learned material to affect material already acquired. This would collectively provide a more comprehensive and reliable assessment of cognitive performance which is influenced by the hippocampus.

#### **7.5.4 Compliance and Basal Cortisol Profiling**

The use of saliva samples for cortisol measurement is a practical and feasible method of exploring basal diurnal cortisol profiles in a free-living paradigm. However, the accuracy and reliability of the profiles obtained is often confounded by the issue of volunteer compliance (Kudielka et al., 2003). Safeguards were employed to preserve the accuracy of the saliva samples collected as previously discussed in the respective Chapters. These included verbal and written instructions for the production of saliva samples for research used in Chapter's Four, Five and Six.

One of the most interesting findings of this thesis was the high prevalence of a non-classic diurnal profile observed throughout the thesis and the psychological and metabolic factors that the incidence of this type of profile appeared to be associated with. This non-classic profile was characterised by a lack of a cortisol awakening response and normal or elevated diurnal cortisol secretion. In the past, researchers have sought to remove individuals not displaying a clear cortisol awakening response from the data set as a means to protect against volunteer non-compliance. However,

approximately 10% of individuals fail to exhibit a cortisol awakening response (Prüssner et al., 1997). Therefore, this may result in exclusion of volunteers with a disrupted HPA axis. If a lack of a cortisol awakening response is a sign of compliance then in the current study, then 48% of the sample in Chapter Five and 47% of the sample in Chapter Six would have been excluded. The findings of this thesis support the argument that a flattened profile is more than just a signal of volunteer non-compliance (Bjorntorp and Rosmond, 2000; Rosmond et al., 1998). Further, the observation that in both Figure 5.6 in Chapter Five and Figure 6.28 in Chapter Six, mean cortisol at waking did not differ significantly between the classic and non-classic groups, effectively eliminates the possibility of non-compliance. Non-classic profiles were associated with a significantly greater incidence of metabolic syndrome symptomology, perceived stress and poor sleep quality. Rivera and Svec (1989) have argued against the exclusion of subgroups in volunteer samples for example, individuals bordering on diabetic in an assessment of cortisol, central obesity and the metabolic syndrome may be an exclusion of the subgroup of most interest. The exclusion of such individuals based on flattened profiles or metabolic conditions may cause a skewness of the characteristics of the group towards those with lower body proportions (peripheral obesity).

## **7.6 Future Research**

The potential for further research to arise as a result of this thesis is great. Initial suggestions are based on suggested improvements to the current studies. For example, of key interest would be an assessment of urinary cortisol output in those with central obesity to elucidate the HPA dysregulation hypothesis. HPA responsivity to stress should be further assessed in those with central obesity in a much larger sample and with pre-test baseline measures of cognitive function across a wide set of cognitive domains to comprehensively assess cognition. Further, gender differences in the response to stress could be explored further concurrent with the assessment of male and female sex hormones, specifically, testosterone and oestrogen. Recent animal research suggests that activation of oestrogen receptors using a selective oestrogen receptor beta agonist improves learning and memory (Day et al., 2006). As discussed previously, centrally obese males have been found to show lower levels of testosterone (Derby et al., 2006) and that testosterone can inhibit HPA activity, particularly the release of ACTH (Williamson et al., 2005). A lower level of testosterone in centrally obese males

could signal increased responsiveness to stress, which would concur with the current findings. Further research to explore the collective effects of sex hormones on stress responsivity and cognition could therefore, be conducted.

Poor diet and physical inactivity are often associated with being chronically stressed, leading to weight gain (Wardle and Gibson, 2002). This may be an alternative route to the expression of the metabolic syndrome (Epel et al., 2004). Building on these observations it would be of interest to explore the potential for diet and exercise to modulate both basal and stimulated cortisol activity in conjunction with the expression of central obesity and the metabolic syndrome. Dimitrov and Koeva (2006) observed a reduction in obesity co-morbidities (triglycerides, LDL) following 3 months of dietary supplementation with omega-3 fatty acid, magnesium and soybean isoflavones. Further, despite no change in body weight or cortisol, better mood and stress coping were observed.

Improvements in blood pressure responses to stress have been observed following a period of aerobic exercise (Brownley et al., 2003; Hamer et al., 2006; Probst et al., 1997). Further, Traustadóttir et al. (2005) observed that greater HPA reactivity marked by increases in cortisol were more common in unfit women. Higher aerobic fitness was associated with a blunted cortisol response to stress. It would be of interest to explore the impact of exercise on stress responsivity particularly in those with central obesity. Further, the impact this could have on the development of metabolic syndrome could be explored. Green et al. (2004) assessed the impact of exercise on metabolic syndrome status in a sample of postmenopausal women with and without oestrogen replacement therapy (ERT) but failed to find an effect. Therefore, there is scope for further research. Exploring the impact of exercise on stress responsivity could also contribute to a test of the hypothesis that the relationship between cortisol and central obesity is mediated through inflammation, based on the observation that weight loss reduces the concentration of inflammatory markers present (Black 2006).

Interestingly, exercise has also been found to influence cognition, particularly in the elderly (Fratiglioni et al., 2004; Van Gelder et al., 2004). In a meta-analysis, Colcombe and Kramer (2003) found evidence to suggest that aerobic training in older persons improves cognitive functioning, particularly executive control. A review by Eggermont

et al. (2006) observed that these improvements were observed even in the presence of cardiovascular risk factors. This implies, that improvements in cognitive function could be observed in individual's borderline for the diagnosis of metabolic syndrome. The main aim of future research might be intervention and prevention, once clear differences have been established in a replication of the studies presented in Chapters Five and Six of this thesis with larger sample sizes.

More interestingly, more recent research has identified a potential relationship between vascular endothelial growth factor (VEGF) and obesity status (Hubold et al., 2006). VEGF has been shown to allocate glucose to the brain (across the blood brain barrier). Based on this Hubold et al. (2006) demonstrated that lower VEGF was linked with the presence of obesity and postulated that this may be linked to the glucose feedback mechanism of food intake and body mass. The relationship between VEGF and cortisol is yet to be determined. However, in terms of the current thesis, it would be interesting to explore the impact lowered VEGF has on cognition, based on the hypothesis that low VEGF reduces brain glucose transportation with consequences for cognitive performance. This may be an alternative mechanism of action to explain poorer cognitive performance in those with central obesity.

### **7.7 Concluding Remarks**

In conclusion, the research presented in this thesis suggests that individuals with central obesity exhibit altered basal cortisol levels compared with lean or peripherally obese individuals. Observed variability in the profiles (and possible pathological cortisol secretion) was associated with a vulnerability to the metabolic syndrome with a flattened profile associated with biological markers of the metabolic syndrome, including elevated inflammatory markers and insulin resistance. The identification of psychological and metabolic parameters associated with the expression of non-classic basal cortisol profiles has important implications for the issue of volunteer compliance. The collective presence of elevated cortisol responses to stress in individuals with central obesity and associated metabolic symptomology appeared to influence cognitive performance leading to impairments on some hippocampal related memory tasks. With further research, these findings could contribute to a more detailed exploration of the impact of chronic stress on psychological and metabolic functioning.

---

---

**References**

---

---

- Abell, T. L., Malagelada, J. R. and Lucas, A. R. (1987). "Gastric electromechanical and neurohormonal function in anorexia nervosa." Gastroenterology **93**: 958-963.
- Abelson, J. L., Liberzon, I., Khan, S., Briggs, H. and Young, E. (2006). Acute cognitive manipulation of human HPA response to pharmacological activation. 37th Annual ISPNE Conference: Hormones and Brain from Cloning to Clinic, Leiden, The Netherlands, In Press.
- Abercrombie, H. C., Giese-Davis, J., Sephton, S., Epel, E. S., Turner-Cobb, J. M. and Spiegel, D. (2004). "Flattened cortisol rhythms in metastatic breast cancer patients." Psychoneuroendocrinology **29**(8): 1082-92.
- Aitkin, R. (1969). "Measurement of feelings using visual analogue scales." Proceedings of Royal Society of Medicine **62**: 989-993.
- Akana, S. F., Scribner, K. A., Bradbury, M. J., Strack, A. M., Walker, C. D. and Dallman, M. F. (1992). "Feedback sensitivity of the rat hypothalamic-pituitary adrenal axis and its capacity to adjust to exogenous corticosterone." Endocrinology **131**: 585-594.
- Albiston, A. L., Obeyesekere, V. R., Smith, R. E. and Krozowski, Z. S. (1994). "Cloning and tissue distribution of the human 11 $\beta$  - Hydroxysteroid Dehydrogenase type 2 isoform." Mol Cell Endocrinol **105**: R11-17.
- Alderson, A. L. and Novack, T. A. (2002). "Neurophysiological and clinical aspects of glucocorticoids and memory: A Review." Journal of Clinical and Experimental Neuropsychology **24**(3): 335-355.
- Anderson, A. K. and Phelps, E. A. (2001). "Lesions of the human amygdala impair enhanced perception of emotionally salient events." Nature **411**(6835): 305-309.
- Andrew, R., Phillips, D. I. W. and Walker, B. (1998). "Obesity and gender influence cortisol secretion and metabolism in man." Journal Clinical Endocrinol Metabolism **83**: 1806-1809.
- Andrews, R. C., Herlihy, O., Livingstone, D. E. W., Andrew, R. and Walker, B. (2002). "Abnormal cortisol metabolism and tissue sensitivity to cortisol in patients with glucose intolerance." Journal Clinical Endocrinology & Metabolism **87**: 5587-5593.
- Backhaus, J., Junghanns, K. and Hohagen, F. (2004). "Sleep disturbances are correlated with decreased morning awakening salivary cortisol." Psychoneuroendocrinology **29**: 1184-1191.

- Bahr, V., Pfeiffer, A. F. and Diederich, S. (2002). "The metabolic syndrome X and peripheral cortisol synthesis." Clinical Endocrinology **110**(7): 313-318.
- Bailey, S. L. and Heitkemper, M. M. (1991). "Morningness-Eveningness and early-morning salivary cortisol levels." Biological Psychology **32**: 181-192.
- Bamberger, C. M., Schultz, H. M. and Chrousos, G. P. (1996). Endocrinology Review **17**: 245-261.
- Bardwell, W. A., Ziegler, M. G. and Dimsdale, J. E. (2000). "Influence of Cholesterol and Fasting Insulin Levels on Blood Pressure Reactivity." Psychosomatic Medicine **62**: 569-575.
- Barrett-Connor, E. and Goodman-Gruen, D. (1999). "Cognitive function and endogenous sex hormones in older women." Journal American Geriatrics **47**: 1289-1293.
- Bebbington, P. (1996). "The origin of sex differences in depressive disorder: bridging the gap." International Review of Psychiatry **8**: 295-332.
- Beckwith, B. E., Petros, T. V., Scaglione, C. and Nelson, J. (1986). "Does-dependent effects of hydrocortisone on memory in human males." Physiological Behaviour **36**: 283-286.
- Bengtsson, B. A., Eden, S. and Lonn (1993). "Treatment of Adults with growth hormone deficiency with recombinant human growth hormone." Journal Clinical Endocrinol Metabolism **76**: 309-317.
- Bent, N., Rabbitt, P. and Metcalfe, D. (2000). "Diabetes Mellitus and the rate of cognitive aging." British Journal Clinical Pharmacology **39**: 349-362.
- Benton, D., Owens, D. S. and Parker, P. Y. (1994). "Blood glucose influences memory and attention in young adults." Neuropsychologia **32**: 595-607.
- Berg, A. H., Combs, T. P., Du, X., Brownlee, M. and CScherer, P. E. (2001). "The adipocyte-secreted protein Acrp30 enhance hepatic insulin action." Natural Medicine **7**: 947-53.
- Bernet, F., Dedieu, J. F., Laborri, C., Montel, V. and Dupouy, J. P. (1998). "Circulating neuropeptide Y (NPY) and catecholamines in rats under resting and stress conditions." Neuroscience Letters **250**: 45-48.
- Bierwolf, C., Struve, K., Marshall, L., Born, J. and Fehm, H. L. (1997). "Slow wave sleep drives inhibition of pituitary-adrenal secretion in humans." Neuroendocrinology **9**: 479-484.
- Bjorntorp, P. (1991). "Visceral fat accumulation: the missing link between psychosocial factors and cardiovascular disease?" Journal International Medicine **230**: 195-201.
- Bjorntorp, P. (1995). "Endocrine Abnormalities of Obesity." Metabolism **44**(9 Suppl.

3): 21-23.

Bjorntorp, P. (1997). "Obesity." Lancet **350**: 423-426.

Bjorntorp, P. (1997). "Body fat distribution, insulin resistance, and metabolic diseases." Nutrition **9**: 795-803.

Bjorntorp, P. and Rosmond, R. (1999). "Perturbations of the hypothalamic-pituitary-adrenal axis and the Metabolic syndrome in ageing." Growth Hormone & IGF Research **9**: 121-123.

Bjorntorp, P. and Rosmond, R. (2000). "Neuroendocrine abnormalities in visceral obesity." International Journal Obesity Related Metabolism Disorders **24**(Suppl.): S90-S85.

Bjorntorp, P. and Rosmond, R. (2000). "Obesity and Cortisol." Nutrition **16**: 924-936.

Bjorntorp, P., Rosmond, R. and Folkow, B. (2000). "Hypertension and the metabolic syndrome: Closely related central origin?" Blood Pressure **9**(2-3): 71-82.

Bjorntorp, P. and Rosmond, R. (2001). "The metabolic syndrome - a neuroendocrine disorder?" British Journal of Nutrition **83**: S49-S57.

Black, P. H. (2003). "The inflammatory response is an integral part of the stress response: Implications for atherosclerosis, insulin resistance, type II diabetes and metabolic syndrome X." Brain Behaviour and Immunity **17**(5): 350-364.

Black, P. H. (2006). "The inflammatory consequences of psychological stress: Relationship to insulin resistance, obesity, atherosclerosis and diabetes mellitus, type II." Medical Hypotheses **In Press**.

Blair, D., Habicht, J. P., Sims, E. A., Sylwester, D. and Abrahan, S. (1984). "Evidence for an increased risk of hypertension with centrally located body fat and the effect of race and sex on this." American Journal Epidemiology **119**: 526-540.

Blascovich, J. and Tomaka, J. (1996). "The Biopsychosocial model of arousal regulation." Advances in Experimental Social Psychology **28**: 1-51.

Bohnen, N., Houx, P., Nicolson, N. and Jolles, J. (1990). "Cortisol Reactivity and cognitive performance in a continuous mental task paradigm." Biological Psychiatry **31**: 107-116.

Bohnen, N., Nicolson, N., Sulon, J. and Jolles, J. (1991). "Coping style, Trait anxiety and cortisol reactivity during mental stress." Journal of Psychosomatic Research **35**(2/3): 141-147.

Bond, A. and Lader, M. (1974). "The Use of Visual Analogue Scales in rating Subjective Feelings." British Journal of Psychology **47**: 211-218.

Borkan, G. A., Gerzof, S. G., Robbins, A. H., Hulst, D. E., Silbert, C. K. and Silbert, J. E. (1982). "Assessment of abdominal fat content by computed tomography." American

Journal of Clinical Nutrition **36**: 172-177.

Born, J., Spath-Schwalbe, E., Schwakenhofer, H., Kern, W. and Fehm, H. L. (1989). "Influences of corticotrophin-releasing hormone (CRH), adrenocorticotrophin (ACTH) and cortisol on sleep in normal man." Clinical Endocrinology Metabolism **68**: 904-911.

Born, J., Ditschuneit, I., Schreiber, M., Dodt, C. and Fehm, H. L. (1995). "Effects of age and gender on Pituitary-adrenocortical responsiveness in humans." European journal of Endocrinology **132**: 705-711.

Born, J. and Fehm, H. L. (1998). "Hypothalamus-pituitary-adrenal activity during human sleep: a coordinating role for the limbic hippocampal system." Clinical Endocrinology **106**: 153-163.

Born, J., Hansen, K., Marshall, L., Molle, M. and Fehm, H. L. (1999). "Timing the end of nocturnal sleep." Nature **397**: 29-30.

Brandenberger, G., Follenius, M. and Hietter, B. (1982). "Feedback from meal-related peaks determines diurnal changes in cortisol response to exercise." Clinical Endocrinology Metabolism **54**: 592-596.

Brandenberger, G., Buguet, A., Spiegel, K., Stanghellini, A., Muanga, G., Bogui, P. and Dumas, M. (1996). "Disruption of endocrine rhythms in sleeping sickness with preserved relationship between hormonal pulsatility and the REM-nREM sleep cycles." Journal Biological Rhythms **11**(3): 258-267.

Breslau, N., Davis, G. C., Andreski, P., Peterson, E. L. and Schultz, L. R. (1997). "Sex differences in posttraumatic stress disorder." Archives of General Psychiatry **54**: 1044-1048.

Brownley, K. A., Hinderlitter, A. L., West, S. G., Girdler, S. S., Sherwood, A. and Light, K. A. (2003). "Sympathoadrenergic mechanisms in reduced haemodynamic stress responses after exercise." Medicine and Science in Sports and Exercise **35**: 978-986.

Buijs, R. M., Kalsbeek, A., Vanderwoude, T. P., Van Heerikhuize, J. J. and Shinn, S. (1993). "Suprachiasmatic nucleus lesion increases corticosterone secretion." American Journal Physiology **264**: 1186-1192.

Buijs, R. M., Wortel, J., Van Heerikhuize, J. J. and Kalsbeek, A. (1997). "Novel environment induced inhibition of corticosterone secretion: Physiological evidence for a Suprachiasmatic nucleus mediated neuronal hypothalamo-adrenal cortex pathway." Brain Research **758**: 229-236.

Buijs, R. M., Hermes, M. and Kalsbeek, A. (1998). The Suprachiasmatic nucleus-Paraventricular nucleus interactions: A bridge to the neuroendocrine and automatic nervous system. Advances in brain Vasopressin: 365-382.

Buijs, R. M., Wortel, J., Van Heerikhuize, J. J., Feenstra, M. G. P., Ter Horst, G. J., Romijn, H. J. and Kalsbeek, A. (1999). "Anatomical and functional demonstration of a multisynaptic Suprachiasmatic nucleus adrenal (cortex) pathway." European journal Neuroscience **11**: 1535-1544.



- Bujalska, I. J., Kumar, S. and Stewart, P. M. (1997). "Does central obesity reflect "Cushing's disease of the omentum"?" The Lancet **349**: 1210-1213.
- Burgess, L. H. and Handa, R., J. (1992). "Chronic oestrogen-induced alterations in adrenocorticotrophin and corticosterone secretion, and glucocorticoid receptor-mediated functions in female rats." Endocrinology **131**: 1261-1269.
- Buros, O. K. (1978). The eighth mental measurements yearbook. New Jersey, Gryphon Press.
- Cacioppo, J. T., Malarkey, W. B., Kiecolt-Glaser, J. K., Uchino, B. N., Sgoutas-Emeh, S. A., Sheridan, J. F., Berntson, G. G. and Glaser, R. (1995). "Cardiac autonomic substrates as a novel approach to explore heterogeneity in neuroendocrine and immune responses to brief psychological stressors." Psychosomatic Medicine **57**: 154-164.
- Cannon, W. B. (1928). "Organisation for the physiological homeostasis." Physiological Review **9**: 399.
- Carlson, L. E. and Sherwin, B. B. (1999). "Steroid hormones, memory and mood in a healthy elderly population." Psychoneuroendocrinology **23**: 583-603.
- Cella, L. K., Van Cauter, E. and Schoeller, D. A. (1995). "Diurnal rhythmicity of human cholesterol synthesis: normal pattern and adaptation to simulated "jet lag"." American Journal Physiology **269**: E489-498.
- Ceresini, G., Morganti, S., Rebecchi, I., Freddi, M., Ceda, G. P., Banchini, A., Solerte, S. B., Ferrari, E., Ablondi, F. and Valenti, G. (2000). "Evaluation of the circadian profiles of serum dehydroepiandrosterone (DHEA) cortisol and cortisol/DHEA molar ration after a single oral administration of DHEA in elderly subjects." Metabolism **49**(4): 548-551.
- Chrousos, G. P. and Gold, P. W. (1992). "The concepts of stress and stress system disorders. Overview of physical and behavioural homeostasis." Journal of the American Medical Association **267**: 1244-1252.
- Clark, L. D., Bauer, W. and Cobb, S. (1952). "Preliminary observations on mental disturbances occurring in patients under therapy with cortisone and ACTH." New England journal of medicine **246**: 205-216.
- Clow, A., Thorn, L., Evans, P. and Hucklebridge, F. (2004). "The awakening cortisol response: Methodological issues and significance." Stress **7**(1): 29-37.
- Cohen, S., Kamarck, T. and Mermelstein, R. (1983). "A global measure of perceived stress." Journal of Health and Social Behaviour **24**: 385-396.
- Cohen, S. (1994). Perceived stress scale. California, Palo Alto.
- Cohn, S. (1987). New concepts of body composition. Vivo body composition studies. K. J. Ellis, S. Yasumura and W. D. Morgan. London, The institute of physical sciences in medicine: 1-14.

- Colcombe, S. and Kramer, A. F. (2003). "Fitness effects on the cognitive function of older adults: a meta-analytic study." Psychological Science **14**: 125 - 130.
- Collins, A. and Frankenhaeuser, M. (1978). "Stress responses in male and female engineering students." Journal Human Stress **4**(2): 43-48.
- Contreras, L. N., Arregger, A. L., Persi, G. G., Gonzalez, N. S. and Cardoso, E. M. (2004). "A new less-invasive and more informative low-dose ACTH test: salivary steroids in response to intramuscular corticotrophin." Clinical Endocrinology **61**: 675-682.
- Convit, A. (2005). "Links between cognitive impairment in insulin resistance an explanatory model." Neurobiology of Aging **26S**: S31-S35.
- Cook, N. J., Schaefer, A. L., Lepage, S. D. M. and Jones, J. (1997). "Radioimmunoassay for cortisol in pig saliva and serum." Agric. Food Chem. **45**: 395-399.
- Craft, S. (2005). "Insulin resistance syndrome and Alzheimer's disease: age and obesity related effects on memory, amyloid, and inflammation." Neuro-Biology of Aging **26S**: S65- S69.
- Crawford, J. R. (1989). Estimation of pre-morbid intelligence: a review of recent developments. Developments in clinical and experimental. J. R. C. a. D. M. Parker. New York, Plenum Press: 55-74.
- Crawford, J. R., Allan, K. M., Cochrane, R. H. B. and Parker, D. M. (1990). "Assessing the validity of NART - estimated pre-morbid IQs in the individual case." British Journal Clinical Psychology **29**: 435-436.
- Cunningham, J. J., Calles-Escandon, J., Garrido, F., Carr, D. B. and Bode, H. H. (1986). "Hypercorticotenuria and diminished pituitary responsiveness to CRF in obese Zucker rats." Endocrinology **118**: 98.
- Dallman, M. F. (1993). "Adaptation of the hypothalamic-pituitary-adrenal axis to chronic stress." Trends in Endocrinology and Metabolism **4**: 62-69.
- Day, M., Arias, R., Zhang, G., Mervis, R., Sung, A., Warwick, H. M., McIlvain, B., Hirst, W., Reinhart, P., Pangalos, M., Marquis, K. and Liu, F. (2006). Oestrogen receptor beta agonists improve memory, synaptic plasticity and activate creb signalling pathways. 37th Annual International Society for Psychoneuroendocrinology (ISPNE) Conference: Hormones and Brain from Cloning to Clinic, Leiden, The Netherlands.
- De Kloet, E. R. and Reul, J. M. (1987). "Feedback action and tonic influence of corticosteroids on brain function: a concept arising from the heterogeneity of brain receptor systems." Psychoneuroendocrinology **12**: 83-105.
- De Kloet, E. R., Vreugdenhil, E., Oitzl, M. S. and Joels, M. (1998). "Brain corticosteroid receptor balance in health and disease." Endocrinology Review **19**: 269-301.

- De Kloet, E. R., Oitzl, M. S. and Joels, M. (1999). "Stress and cognition: are corticosteroids good or bad guys?" Trends Neuroscience **22**: 422-426.
- de Leon, M. J., McRae, T., Tsai, J. R., George, A. E., Marcus, D. L., Freedman, M. R., Wolf, A. P. and McEwen, B. (1987). "Abnormal cortisol response in Alzheimer's disease linked to hippocampal atrophy." Lancet **2**: 391-392.
- de Leon, M. J., Rusinek, M., R.H., de Santi, S., Convit, A., Tarshish, C., Golomb, J., Volkow, N., Daisley, K., Orentreich, N. and McEwen, B. (1997). "Cortisol reduces hippocampal glucose metabolism in normal elderly, but not in Alzheimer's disease." J. Clin Endocrinol Metab **82**: 3251-9.
- De Luca, C. R., Wood, S. J., Anderson, V., Buchanan, J. A., Proffitt, T. M., Mahony, K. and Pantelis, C. (2003). "Normative data from the Cantab. I: Development of executive function over the lifespan." Journal of Clinical and Experimental Neuropsychology **25**(2): 242-254.
- De Pergola, G., Giagulli, V. A. and Garruti, G. (1991). "Low dehydroepiandrosterone circulating levels in pre-menopausal obese women with very high body mass index." Metabolism **40**: 187-190.
- De Pergola, G., Zamboni, M. and Sciaraffia, M. (1996). "Body fat accumulation is possibly responsible for lower dehydroepiandrosterone circulating levels in pre-menopausal obese women." International Journal of Obesity **20**: 1105-1110.
- De Quervain, D. J. F., Roozendaal, B. and McGaugh, J. L. (1998). "Stress and glucocorticoids impair retrieval of long-term spatial memory." Nature **394**: 787-790.
- De Quervain, D. J. F., Roozendaal, B. and McGaugh, J. L. (2000). "Acute cortisone administration impairs retrieval of long term declarative memory in humans." Nature Neuroscience **3**: 313-314.
- De Souza, E. B. and Van Loon, G. R. (1982). "Stress-induced inhibition of the plasma corticosterone response to a subsequent stress in rats: A non-adrenocorticotrophin-mediated mechanism." Endocrinology **110**: 23.
- De Souza, E. B. and Van Loon, G. R. (1986). "Brain serotonin and catecholamine responses to repeated stress in rats." Brain Research **367**: 77-86.
- De Vente, W., Olf, M., Van Amsterdam, J. G. C., Kamphuis, J. H. and Emmelkamp, P. M. G. (2003). "Physiological differences between burnout patients and healthy controls: blood pressure, heart rate, and cortisol responses." Occupational Environmental Medical **60**: i54 - i61.
- DeFronzo, R. A. and Ferrannini, E. (1991). "Insulin resistance: A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease." Diabetes Care **14**: 173-194.
- Derby, C. A., Zilbert, S., Brambillat, D., Knashawn, H. and McKinlay, J. B. (2006). "Body mass index, waist circumference and waist to hip ratio and change in sex steroid

- hormones: the Massachusetts male ageing study." Clinical Endocrinology **65**: 125-131.
- Desai, H. D. and Jann, M. W. (2000). "Major depression in women: a review of the literature." Journal American Pharmacology Association **40**: 525-537.
- Dettenborn, L., James, G.D., van Berge-Landry, H., Valdimarsdottir, H. B., Montgomery, G. H. and Bovbjerg, D. H. (2005). "Heightened Cortisol responses to daily stress in working women at familial risk to breast cancer." Biological Psychology **69**: 167-179.
- Deurenberg, P., Weststrate, J. A. and Seidell, J. C. (1991). "Body mass index as a measure of body fatness: age and sex specific prediction formulas." British Journal Nutrition **65**: 105-114.
- Dickerson, S. S. and Kemeny, M. E. (2004). "Acute Stressors and Cortisol Responses: A Theoretical Integration and Synthesis of Laboratory Research." Psychological Bulletin **130**(3): 355-391.
- Dienstbier, R. A. (1989). "Arousal and physiological toughness: Implications for mental and physical health." Psychological Review **96**: 84-100.
- Dijkstra, I., Binnekade, R. and Tilders, F. J. H. (1996). "Diurnal variation in resting levels of corticosterone is not mediated by variation in adrenal responsiveness to adrenocorticotrophin but involves splanchnic nerve integrity." Endocrinology **137**: 540-547.
- Dimitrov, D. and Koeva, L. (2006). Dietary supplements for stress and obesity comorbidities reduction. 37th Annual International Society for Psychoneuroendocrinology (ISPNE) Conference: Hormones and Brain from Cloning to Clinic, Leiden, The Netherlands.
- Domes, G., Heinrichs, M., Reichwald, U. and Hautzinger, M. (2002). "Hypothalamic-pituitary-adrenal axis reactivity to psychological stress and memory in middle aged women: high responder's exhibit enhanced declarative memory performance." Psychoneuroendocrinology **27**: 843-853.
- Doyle, P., Rohner-Jeanrenaud, F. and Jeanrenaud, B. (1993). "Local cerebral glucose utilisation in brains of lean and genetically obese (fa/fa) rats." Brain Research **264**: 29-35.
- Drafta, D., Schindler, A. E., Stroe, E. and Neacsu, E. (1982). "Age-related changes of plasma steroids in normal adult males." Journal Steroid Biochemistry **17**: 683-687.
- Dubuc, P. U. and Wilden, N. J. (1986). "Adrenalectomy reduces but does not reverse obesity in OB/OB mice." International Journal Obesity **10**: 91-98.
- Duclos, M., Corcuff, J. B., Etchjevery, N., Rashedi, M., Tabarin, A. and Roger, P. (1999). "Abdominal obesity increases overnight cortisol excretion." Journal of Endocrinological investigation **22**(6): 465-471.
- Durnin, J. V. G. A. and Rahaman, M. M. (1967). "The assessment of the amount of fat

- in the human body from measurements of skin fold thickness." British Journal Nutrition **21**: 681-689.
- Dye, L. and Blundell, J. (2002). "Functional foods: psychological and behavioural functions." British Journal of Nutrition **88**(6): 181-211.
- Edwards, C. R. W., Stewart, P. M., Nairn, I. M., Grieve, J. and Shackelton, C. H. L. (1985). "Cushing's disease of the kidney." Endocrinology **104s**(53).
- Edwards, S., Evans, P., Hucklebridge, F. and Clow, A. (2001). "Association between time of awakening and diurnal cortisol secretory activity." Psychoneuroendocrinology **26**: 613-622.
- Edwards, S., Clow, A., Evans, P. and Hucklebridge, F. (2001). "Exploration of awakening cortisol response in relation to diurnal cortisol secretory activity." Life Sciences **68**: 2093-2103.
- Edwards, S., Hucklebridge, F., Clow, A. and Evans, P. (2003). "Components of the Diurnal cortisol cycle in relation to upper respiratory symptoms and perceived stress." Psychosomatic Medicine **65**: 320-327.
- Eggermont, L., Swaab, D., Luiten, P. and Scherder, E. (2006). "Exercise, cognition and Alzheimer's disease: More is not necessarily better." Neuroscience and Biobehavioural reviews **30**: 562-575.
- Elias, M. F., Wolf, P. A., D'Agostino, R. B., Cobb, S. and White, L. R. (1993). "Untreated blood pressure level is inversely related to cognitive functioning: the Framingham study." American Journal Epidemiology **138**: 353-364.
- Elias, M. F., D'Agostino, R. B., Elias, P. K. and Wolf, P. A. (1995). "Blood pressure, hypertension and age as risk factors for poor cognitive performance." Exp Aging Res **21**: 393-417.
- Elias, M. F., D'Agostino, R. B., Elias, P. K. and Wolf, P. A. (1995). "Neuropsychological test performance, cognitive functioning, blood pressure, and age: The Framingham Heart Study." Exp Aging Res **21**: 369-391.
- Elias, P. K., Elias, M. F., D'Agostino, R. B., Cupples, L. A., Wilson, P. W., Silbershatz, H. and Wolf, P. A. (1997). "NIDDM and blood pressure as risk factors for poor cognitive performance. The Framingham study." Diabetes Care **20**: 1388-1395.
- Elias, M. F., Elias, P. K., Sullivan, L. M., Wolf, P. A. and D'Agostino, R. B. (2005). "Obesity, diabetes and cognitive deficit: The Framingham Heart Study." Neurobiology of Aging **26s**: S11-S16.
- Elliott, D. (1993). "Comparison of three instruments for measuring patient anxiety in coronary care unit." Intensive Critical Care Nursing **9**: 195-200.
- Elzinga, B. M., Bakker, A. and Bremner, J. D. (2005). "Stress-induced cortisol elevations are associated with impaired delayed, but not immediate recall." Psychiatry Research **134**: 211-223.

- Elzinga, B. M. and Roelofs, K. (2005). "Cortisol-induced impairments of working memory require acute sympathetic activation." Behavioural Neuroscience **119**: 98-103.
- Epel, E. S., McEwen, B., Seeman, T. E., Matthews, K., Castellazzo, G., Brownell, K. D., Bell, J. and Ickovics, J. (2000). "Stress and body shape: Stress-induced cortisol secretion is consistently greater among women with central fat." Psychosomatic Medicine **62**: 623-632.
- Epel, E. S., Jimenez, S., Brownell, K. D., Stroud, L., Stoney, C. and Niaura, R. (2004). "Are stress eaters at risk for metabolic syndrome?" Annals of New York Academy of Science **1032**: 208-210.
- Everson, S. A., Lynch, J. W., Kaplan, G. A., Lakka, T. A., Sivenius, J., Salonen, J. T. and Matthews, K. A. (2001). "Stress-Induced Blood Pressure Reactivity and Incident Stroke in Middle-Aged Men Editorial Comment: Something Old and Something New." Stroke **32**(6): 1263-1270.
- Federenko, I., Wüst, S., Hellhammer, D., Dechoux, R., Kumsta, R. and Kirschbaum, C. (2004). "Free cortisol awakening responses are influenced by awakening time." Psychoneuroendocrinology **29**: 174-184.
- Fehm, H. L., Klien, E., Holl, R. and Voigt, K. H. (1984). "Evidence for extra-pituitary mechanisms mediating the morning peak of plasma-cortisol in man." Clinical Endocrinology Metabolism **58**: 410-414.
- Fehm-Wolfsdorf, G., Reutler, K., Zenz, H., Born, J. and Fehm, H. L. (1993). "Are circadian variations in taste thresholds cortisol dependent?" Journal Psychophysiology **7**: 65-72.
- Feldman, D. (1978). "Evidence that brown adipose tissue is a glucocorticoid target organ." Endocrinology **103**: 2091-2097.
- Fernandez-Real, J. M., Pugeat, M., Grasa, M., Broch, M., Vendrell, J., Brun, J. and Ricart, W. (2003). "Serum corticosteroid-binding globulin concentration and insulin resistance syndrome: a population study." Clinical Endocrinology Metabolism **87**: 4686-4690.
- Field, A. E., Colditz, G. A., Willett, W. C., Longcope, C. and McKinlay, J. B. (1994). "The relation of smoking, age, relative weight, and dietary intake to serum adrenal steroids, sex hormones, and sex hormone-binding globulin in middle-aged men." Journal Clinical Endocrinol Metabolism **79**: 1310-1316.
- Foley, D. J., Monjan, A. A., Brown, S. L., Simonsick, E. M., Wallace, R. B. and Blazer, D. G. (1995). "Sleep complaints among older persons: an epidemiological study of three communities." Sleep **18**: 425-432.
- Foley, D. J., Monjan, A. A., Simonsick, E. M., Wallace, R. B. and Blazer, D. G. (1999). "Incidence and remission of insomnia among elderly adults an epidemiologic study of 6'800 persons over three years." Sleep **122**(2): 366-372.

- Follenius, M., Brandenberger, G., Bandesapt, J. J., Libert, J. P. and Ehrhart, J. (1992). "Nocturnal cortisol release in relation to sleep structure." Sleep **15**(1): 21-27.
- Forsman, L. and Lundberg, U. (1982). "Consistency in catecholamine and cortisol excretion in males and females." Pharmacology Biochemistry Behaviour **17**(3): 555-562.
- Fowler, K. S., Saling, M. M., Conway, E. L., Semple, J. M. and Louis, W. J. (1997). "Computerized neuropsychological tests in the early detection of dementia: prospective findings." Journal of International Neuropsychological society **3**: 139-146.
- Frankenfield, D. C., Rowe, W. A., Cooney, R. N., Stanley Smith, J. and Becker, D. (2001). "Limits of body mass index to detect obesity and predict body composition." Nutrition **17**: 26-30.
- Frankenhaeuser, M., Rauste von Wright, M., Collins, A., von Wright, J., Sedvall, G. and Swahn, C. G. (1978). "Sex difference in psychoneuroendocrine reactions to examination stress." Psychosom Med **40**: 334-343.
- Fraser, R., Ingram, M. C., Anderson, N. H., Morrison, C., Davies, E. and Connell, J. M. C. (1999). "Cortisol effects in body mass, blood pressure, and cholesterol in the general population." Hypertension **33**: 1364-1368.
- Fratignioni, L., Paillard-Borg, S. and Winblad, B. (2004). "An active and socially integrated lifestyle in late life might protect against dementia." Lancet **3**: 343-353.
- Freedman, M. R., Horwitz, B. A. and Stern, J. S. (1986). "Effect of adrenalectomy and glucocorticoid replacement on development of obesity." American Journal Physiology **249**: R595 - R607.
- Friedman, M., Green, M. F. and Sharland, D. E. (1969). "Assessment of hypothalamic-pituitary-adrenal function in the geriatric age group." Journal Gerontol **24**: 292-297.
- Fries, E., Hellhammer, J. and Hellhammer, D. (2006). Perceived chronic stress impacts on the cortisol response to the Trier Social Stress Test. 37th Annual International Society for Psychoneuroendocrinology (ISPNE) Conference: Hormones and Brain from Cloning to Clinic, Leiden, The Netherlands.
- Friess, E., Tagaya, H., Grethe, C., Trachsel, L. and Holsboer, F. (2004). "Acute Cortisol Administration Promotes Sleep Intensity in Man." Neuropsychopharmacology **29**: 598-604.
- Froelicher, E. S., Li, W. W., Mahrer-Imhof, R., Christopherson, D. and Stewart, A. L. (2004). "Women's initiative for non smoking (WINS) VI: Reliability and validity of health and psychosocial measures in women smokers with cardiovascular disease." Heart and Lung **33**: 162-175.
- Fujimoto, V. Y., Villanueva, A. L., Hopper, B., Moscinski, M. and Rebar, R. W. (1986). "Increased adrenocortical responsiveness to exogenous ACTH in oral contraceptive users." Adv. Contracept **2**: 343-353.

- Fuster, J. M. (1980). The prefrontal cortex. New York.
- Gaab, J., Rohleder, N., Nater, U. M. and Ehlert, U. (2005). "Psychological determinants of the cortisol stress response: the role of anticipatory cognitive appraisal." Psychoneuroendocrinology **30**: 599-610.
- Gale, E. A. M. (2002). "Editorial: the myth of the metabolic syndrome." Diabetologia **10**: 1873-1875.
- Galea, L. A. M., McEwen, B., Tanapat, P., Deak, T., Spencer, R. L. and Dhabhar, F. S. (1997). "Sex differences in dendritic atrophy of CA3 pyramidal neurons in response to chronic restraint stress." Neuroscience **81**: 689-697.
- Gallucci, W. T., Baum, A., Laue, L., Rabin, D. S., Chrousos, G. P., Gold, P. W. and Kling, M. (1993). "Sex differences in sensitivity of the hypothalamic-pituitary-adrenal axis." Health Psychology **12**: 420-425.
- Galvao-Teles, A., Graves, L., Burke, C. W., Fotherby, K. and Fraser, R. (1976). "Free cortisol in obesity; effect of fasting." Endocrinology **81**(2): 321-329.
- Glass, A. R., Burman, K. D., Dahms, W. T. and Boehm, T. M. (1981). "Endocrine function in human obesity." Metabolism **30**: 89-104.
- Gold, P. E. (1987). "Sweet Memories/." Am. Sci **75**: 151-155.
- Goldman, N., Gleib, D. A., Seplaki, C., I-Wen, L. and Weinstein, M. (2005). "Perceived stress and physiological dysregulation in older adults." Stress **8**(2): 95-105.
- Goldstein, F., Chen, J., Wilson, R. S. and Manson, J. E. (2001). "Type 2 diabetes and cognitive function in community-dwelling elderly women." Diabetes Care **24**: 1060-1065.
- Goodyer, I. M., Herbert, J., Altham, P. M. E., Pearson, J., Secher, S. M. and Shiers, H. M. (1996). "Adrenal secretion during major depression in 8 to 16 year olds. 1. Altered diurnal rhythms in salivary cortisol and dehydroepiandrosterone (DHEA) at presentation." Psychology Medicine **33**: 601-610.
- Gotthardt, U., Schweiger, U., Fahrenberg, J., Lauer, C. J., Holsboer, F. and Heuser, I. (1995). "Cortisol, ACTH and cardiovascular response to cognitive challenge paradigm in aging and depression." American Journal of Physiology **268**: R865 - R873.
- Gozansky, W. S., Lynn, J. S., Laudenslagert, M. L. and Kohrt, W. M. (2005). "Salivary cortisol determined by enzyme immunoassay is preferable to serum total cortisol for assessment of dynamic hypothalamic-pituitary-adrenal axis activity." Clinical Endocrinology **63**: 336-341.
- Green, J. S., Stanforth, P. R., Rankinen, T., Leon, A. S., Rao, D. C., Skinner, J. S., Bouchard, C. and Wilmore, J. H. (2004). "The effects of exercise training on abdominal visceral fat, Body composition, and indicators of the metabolic syndrome in postmenopausal women with and without oestrogen replacement therapy: The Heritage family study." Metabolism **53**: 1192-1196.



- Greendale, G. A., Kritz-Silverstein, D., Seeman, T. E. and Barrett-Connor, E. (2000). "Higher basal cortisol predicts verbal memory loss in post-menopausal women: Rancho Bernardo study." Journal American Geriatrics **48**(12): 1655-1658.
- Groop, L., Forsblom, C., Lehtovirta, M., Tuomi, T., Karanko, S., Nissen, M., Ehrnstrom, B. O., Forsen, B., Isomaa, B., Snickars, B. and Taskinen, M. R. (1996). "Metabolic consequences of a family history of NIDDM (The Bosnia Study): evidence or sex specific parental effects." Diabetes Care **45**: 1585-1593.
- Groschl, M., Rauh, M., Wagner, R., Neuhuber, W., Metzler, M., Tamguney, G., Zenk, J., Schoof, E., Dorr, H. G., Blum, W. F., Rascher, W. and Dotsch, J. (2001). "Identification of Leptin in Human Saliva." Journal Clinical Endocrinology & Metabolism **86**(11): 5234-5239.
- Grossi, G., Perski, A., Ekstedt, M., Johansson, T., Lindstrom, M. and Holm, K. (2005). "The morning salivary cortisol response in burnout." Journal of Psychosomatic Research **59**: 103-111.
- Hamer, M., Taylor, A. and Steptoe, A. (2006). "The effect of acute aerobic exercise on stress related blood pressure responses: Asystematic review and meta-analysis." Biological Psychology **71**: 183-190.
- Handa, R., J., Burgess, L. H., Kerr, J. E. and O'Keefe, J. A. (1994). "Gonadal steroid hormone receptors and sex differences in the Hypothalamic-pituitary-adrenal axis." Hormones and Behaviour **28**: 464-476.
- Hansen, A. L., Snow, A. V., Murison, R., Helge Johnsen, B. and Thayer, J. F. (2006). Individual differences in the relationship between cortisol and cognitive function: effects of resting heart rate variability. 37th Annual International Society for Psychoneuroendocrinology (ISPNE) Conference: Hormones and Brain from Cloning to Clinic, Leiden, The Netherlands.
- Hartz, A. J., Rupley, D. C. and Rimm, A. A. (1984). "The association of girth measurements with disease in 32856 women." American Journal Epidemiology **119**: 71-80.
- Hashizume, K., Suzuki, S., Hara, M., Komatsu, A. and Yamashita, K. (2006). "Metabolic syndrome and age-related dementia: Endocrinological aspects of adaptation to aging." Mechanisms of Ageing and Development **127**: 507-510.
- Hauer, H., Schmid, P. and Pfeiffer, E. R. (1987). "Glucocorticoids and insulin promote the differentiation of human adipocyte precursor cells into fat cells." Journal Clinical Endocrinology Metabolism **64**: 832-835.
- Heatherton, T. F. and Polivy, J. (1991). "Development and validation of a scale for measuring state self-esteem." Journal of Personality and social psychology **60**(6): 895-910.
- Hellhammer, J. (1990). Burnout bei Pflegepersonal-eine psychoendokrinologische Untersuchung. Diploma Thesis, University of Trier.

- Hemmilla, I., Dakubu, S., Mikkala, V.-M., Siitari, H. and Lovgren, T. (1984). "Europium as a label in time resolved immunofluorometric assays." Annals of Biochemistry **137**: 335-343.
- Hennig, J., Netter, P. and Voigt, K. H. (2000). "Mechanisms of changes in lymphocyte numbers after psychological stress." Zeitschrift für Rheumatologie **59**: 43-48.
- Henrichs, S. C., Menzaghi, F., Merlo-pich, E., Hanger, R. L. and Koob, G. F. (1993). "Corticotrophin-releasing factor in the Paraventricular nucleus modulates feeding induced by neuropeptide." Brain Research **611**: 18-24.
- Henry, J. P. and Grim, C. E. (1990). "Psychosocial mechanisms of primary hypertension." Journal of Hypertension **8**: 783-793.
- Herbert, M., Johns, M. W. and Dore, C. (1976). "Factor Analysis of analogue scales measuring subjective feelings before and after sleep." British Journal of Medical Psychology **49**(4): 373-9.
- Herbert, T. B. and Cohen, S. (1993). "Stress and immunity in humans: a meta-analytic review." Psychosomatic Medicine **55**: 364-379.
- Herrmann, C., Scholz, K., H, and Kreuzer, H. (1991). "Psychologisches screening von patienten einer kardiologischen Akutklinik mit einer deutschen fassung der "Hospital Anxiety and Depression" (HAD) skala." Psychosomatic Medicine **41**: 83-92.
- Herrmann, C., Buss, U. and Snaith, R. P. (1995). "HADS-D: Hospital Anxiety Depression Scales - Deutsche Version." Verlag Hans Huber.
- Herrmann, C. (1997). "International experiences with Hospital Anxiety and Depression scale - A review of validation data and clinical results." Journal of Psychosomatic Research **42**(1): 17-41.
- Heuser, I., Gotthardt, U., Schweiger, U., Schmider, J., Lammers, C. H., Dettling, M. and Holsboer, F. (1994). "Age-associated changes of the pituitary-adrenocortical hormone regulation in humans: importance of gender." Neurobiology of Aging **15**: 227-231.
- Hiltunen, L. A., Keinänen-Kiukaanniemi, S. M. and Laara, E. M. (2001). "Glucose tolerance and cognitive impairment in an elderly population." Public Health **115**: 197-200.
- Hishinuma, E. S., Miyamoto, R. H., Nishimura, S. T., Goebert, D. A., Yuen, N. Y. C., Makini, G. K., Andrade, N. N., Johnson, R. C. and Carlton, B. S. (2001). "Prediction of anxiety disorders using the state-Trait anxiety inventory for multiethnic adolescents." Anxiety Disorders **15**: 511-533.
- Ho, S. Y., Lam, T. H. and Janus, E. D. (2003). "Waist to stature ratio is more strongly associated with cardiovascular risk factors than other simple anthropometric indices." Annals of Epidemiology **13**(10): 683-691.
- Hoffman, R. and al Absi, M. (2004). "The effect of acute stress on subsequent

- neuropsychological test performance (2003)." Archives of Clinical Neuropsychology **19**: 497-506.
- Holroyd, K. A. and Lazarus, R. S. (1982). Stress, coping and somatic adaptation. Handbook of stress: Theoretical and clinical aspects. L. Goldberger and S. Breznitz, . New York, Free Press.
- Horner, H., Packan, D. R. and Sapolsky, R. (1990). "Glucocorticoids inhibit glucose transport in cultured hippocampal neurons and glia." Neuroendocrinology **52**: 57-63.
- Horrocks, P. M., Jones, A. F., Ratcliff, W. A., Holder, G., White, A., Holder, R., Ratcliff, J. G. and London, D. R. (1990). "Patterns of ACTH and cortisol pulsatility over twenty four hours in normal males and females." Clinical Endocrinology **32**: 127-134.
- Houtkooper, L. B., Lohman, T. G., Going, S. B. and Howell, W. H. (1996). "Why bioelectrical impedance analysis should be used for estimating adiposity." American Journal of Clinical Nutrition **64**(3): S436-S438.
- Hubold, C., Conrad, M., Krueger, H., Oltmans, K. M., Fehm, H. L. and Peters, A. (2006). Obesity is associated with low plasma VEGF concentrations. 37th Annual International Society for Psychoneuroendocrinology (ISPNE) Conference: Hormones and Brain from Cloning to Clinic, Leiden, The Netherlands.
- Hucklebridge, F., Sen, S., Evans, P. D. and Clow, A. (1998). "The relationship between circadian patterns of salivary cortisol and endogenous inhibitor of monoamine oxidase A." Life Sciences **62**: 2321-2328.
- Hucklebridge, F., Clow, A., Abeyguneratne, T., Huezio-Diaz, P. and Evans, P. (1999). "The awakening cortisol response and blood glucose levels." Life Sciences **64**: 931-7.
- Hucklebridge, F., Clow, A., Rahman, H. and Evans, P. (2000). "The cortisol response to normal and nocturnal awakening." Psychophysiology **14**: 24-28.
- Hucklebridge, F., Mellins, J., Evans, P. and Clow, A. (2002). "The awakening cortisol response: no evidence for an influence of body posture." Life Sciences **71**: 639-646.
- Hucklebridge, F., Hussain, T., Evans, P. and Clow, A. (2005). "The diurnal patterns of the adrenal steroids cortisol and dehydroepiandrosterone (DHEA) in relation to awakening." Psychoneuroendocrinology **30**: 51-57.
- Ice, G. H., Katz-Stein, A., Himes, J. and Kane, R. L. (2004). "Diurnal cycles of salivary cortisol in older adults." Psychoneuroendocrinology **29**: 355-370.
- Invitti, C., Redaelli, G., Baldi, G. and Cavagnini, F. (1999). "Glucocorticoid receptors in anorexia nervosa and Cushing's disease." Biol. Psychiatry **45**: 1467-1471.
- Isomaa, B., Almgren, P., Tuomi, T., Forsen, B., Lahti, K., Nissen, M., Taskinen, M. R. and Groop, L. (2001). "Cardiovascular morbidity and mortality associated with the metabolic syndrome." Diabetes Care **683-689**.
- Isomaa, B. (2003). "A major health hazard: The metabolic syndrome." Life Sciences **73**:

2395-2411.

Jacobsen, L. and Sapolsky, R. M. (1991). "The role of the hippocampus in the feedback regulation of the hypothalamic-pituitary-adrenal axis." Endocr. Rev **12**: 118-34.

Jagust, W., Harvey, D., Mungas, D. and Hann, M. (2006). "Central obesity and the aging brain." Archives of Neurology **62**: 1545-1548.

Jamieson, P. M., Chapman, K. E., Edwards, C. R. W. and Seckl, J. R. (1995). "11 $\beta$ -hydroxysteroid dehydrogenase is an exclusive 11 $\beta$ -reductase in primary rat hepatocyte cultures: effect of physiochemical and hormonal manipulations." Endocrinology **136**: 4754-61.

Janis, I. L. and Field, P. B. (1959). Sex differences and factors related to persuasibility. Personality and Persuasibility. C. I. H. a. I.L.Janis. New Haven, Yale University Press.

Jaquet, D., Gaboriau, A., Czernichow, P. and Levy-Marchal, C. (2000). "Insulin resistance early in adulthood in subjects with intrauterine growth retardation." Journal of Clinical Endocrinology and Metabolism **85**: 1401-1406.

Jensen, H. K. and Blichert-Toft, M. (1971). "Serum corticotrophin plasma cortisol and urinary excretion of 17-ketogenic steroids in the elderly (age group 66-94 years)." Acta Endocrinology **66**: 25-34.

Joels, M. and DeKloet, E. R. (1992). "Control of neuronal excitability by corticosteroid hormones." Trends in Neuroscience **15**: 25-30.

Jonker, C., Geerlings, M. I. and Schmand, B. (2000). "Are memory complaints predictive for dementia? A review of clinical and population-based studies." International Journal Geriatric Psychiatry **15**: 983-991.

Kahn, J. P., Rubinow, D. R., Davis, C. L., Kling, M. and Post, R. M. (1988). "Salivary cortisol: a practical method for evaluation of adrenal function." Biological Psychiatry **23**: 335-349.

Kajantie, E., Eriksson, J., Osmond, C., Wood, P. J., Forsen, T., Barker, D. J. and Phillips, D. I. W. (2004). "Size at birth, the metabolic syndrome and 24-h salivary cortisol profile." Clinical Endocrinology Metabolism **60**(2): 201-207.

Kajantie, E. and Phillips, D. I. W. (2006). "The effects of sex and hormonal status on the physiological response to acute psychosocial stress." Psychoneuroendocrinology **31**: 151-178.

Kalimi, S., Shafagoj, Y., Lorai, R., Padgett, D. and Regelson, W. (1994). "Anti-glucocorticoid effects of the dehydroepiandrosterone (DHEA)." Moll Cell Biochem **131**: 99-104.

Kalkhoff, R. K., Hartz, A. H., Rupley, D. C., Kissebah, A. H. and Kelber, S. (1983). "Relationship of body fat distribution to blood pressure, carbohydrate tolerance and plasma lipids in healthy obese women." Journal Lab Clinical Medicine **102**: 621-627.

- Kalmijin, S., Launer, L. J., Stolck, R. P., de Jong, F. H., Pols, H. A., Hofman, A., Breteler, M. M. and Lamberts, S. W. (1998). "A prospective study on cortisol, dehydroepiandrosterone sulfate, and cognitive function in elderly." Clinical Endocrinology Metabolism **83**(10): 3487-3492.
- Kalsbeek, A., Buijs, R. M., Van Heerikhuizen, J. J., Arts, M. and Vanderwoude, T. P. (1992). "Vasopressin-containing neurons of the Suprachiasmatic nuclei inhibit corticosterone release." Brain Research **580**: 62-67.
- Kalsbeek, A., VanderVliet, J. and Buijs, R. M. (1996). "Decrease of endogenous vasopressin release necessary for expression of the circadian rise in plasma corticosterone: A reverse microdialysis study." Neuroendocrinology **8**: 229-307.
- Kanner, A. D., Coyne, J. C., Schaefer, C. and Lazarus, R. S. (1981). "Comparison of two modes of stress measurement: Daily hassles and uplifts versus major life events." Journal of Behavioural Medicine **4**(1): 1-39.
- Katch, F. I. and Katch, V. I. (1984). "The body composition profile: techniques of measurement and applications." Clinical Sports medicine **3**: 31-43.
- Katz, J. R., Taylor, N. F., Goodrick, S., Perry, L., Yudkin, J. S. and Coppack, S. W. (2000). "Central obesity, depression and the hypothalamo-pituitary-adrenal axis in men and postmenopausal women." International Journal Obesity Related Metabolic Disorders **24**: 246-251.
- Keller-Wood, M. E. and Dallman, M. F. (1984). "Corticosteroid inhibition of ACTH secretion." Endocrinology Review **5**: 1-24.
- Kessler, R. C., Brown, R. L. and Broman, C. L. (1981). "Sex differences in psychiatric help seeking: evidence from four large scale surveys." Journal of Health and Social Behaviour **22**: 49-64.
- Kessler, R. C. and McLeod, J. D. (1984). "Sex differences in vulnerability to undesirable life events." American Sociological Review **49**: 620-631.
- Khani, S. and Tayek, J. A. (2001). "Cortisol increase gluconeogenesis in humans: its role in the metabolic syndrome." Clinical Science **101**(6): 739-747.
- Kilander, L., Nyman, H., Boberg, M. and Lithell, H. (1997). "Cognitive function vascular risk factors and education: a cross-sectional study based on a cohort of 70 year old men." Journal of International Medicine **24**: 313-321.
- Kimura, D. (2000). Sex and Cognition. Boston, MIT Press.
- Kirschbaum, C., Wüst, S., Faig, H. G. and Hellhammer, D. H. (1992). "Heritability of cortisol responses to human corticotrophin-releasing hormone, ergometry, and psychological stress in humans." Clinical Endocrinology Metabolism **75**: 1526-1530.
- Kirschbaum, C., Wüst, S. and Hellhammer, D. H. (1992). "Consistent sex differences in cortisol responses to psychological stress." Psychosomatic Medicine **54**: 648-657.

- Kirschbaum, C., Pirke, C. M. and Hellhammer, D. H. (1993). "The "Trier Social Stress Test" - A tool for investigating psychobiological stress responses in a laboratory setting." Neuropsychobiology **28**: 76-81.
- Kirschbaum, C., Prüssner, J. C., Stone, A. A., Federenko, I., Gaab, J., Lintz, D., Schommer, N. and Hellhammer, D. H. (1995). "Persistent high cortisol responses to repeated psychological stress in a subpopulation of healthy men." American Psychosomatic Society **57**(5): 468-474.
- Kirschbaum, C., Wolf, O. T., May, M., Wippich, W. and Hellhammer, D. H. (1996). "Stress and treatment induced elevations of cortisol levels associated with impaired declarative memory in healthy adults." Life Sciences **58**: 1475-83.
- Kirschbaum, C., Bono, E. G., Rohleder, N., Gezzner, C., Pirke, C. M., Salvador, A. and Hellhammer, D. H. (1997). "Effects of fasting and glucose load on free cortisol responses to stress and nicotine." Journal of Clinical Endocrinology and Metabolism **82**: 1101-1105.
- Kirschbaum, C., Kudielka, B. M., Gaab, J., Schommer, N. C. and Hellhammer, D. (1999). "Impact of gender menstrual cycle phase and oral contraceptives on the activity of the hypothalamic-pituitary-adrenal axis." Psychosomatic Medicine **61**(2): 154-162.
- Kontogianni, M. D., Panagiotakos, D. B. and Skopouli, F. N. (2005). "Does body mass index reflect adequately the body fat content in perimenopausal women?" Maturitas **51**: 307-313.
- Kotler, D. P., Burastero, S., Wang, J. and Pierson Jr, R. N. (1996). "Prediction of body cell mass, fat free mass and total body water with bioelectrical impedance analysis: effects of race, sex and disease." American Journal of Clinical Nutrition **64**(suppl.): 489s-497s.
- Kraemer, R. R., Synovitz, L. B., Gimple, T., Kraemer, G. R., Johnson, L. G. and Castracane, V. D. (2001). "Effect of oestrogen on serum DHEA in younger and older women and the relationship of DHEA to adiposity and Gender." 50(488-493).
- Kroenke, K. and Spitzer, R. L. (1998). "Gender differences in the reporting of physical and somatoform symptoms." Psychosomatic Medicine **60**: 150-155.
- Kudielka, B. M., Hellhammer, J., Hellhammer, D. H., Wolf, O. T., Pirke, K. M., Varadi, E., Pilz, J. and Kirschbaum, C. (1998). "Sex differences in endocrine and psychological responses to psychosocial stress in healthy elderly subjects and the impact of a 2-week dehydroepiandrosterone treatment." Clinical Endocrinology Metabolism **83**: 1756-1761.
- Kudielka, B. M., Schmidt-Reinwald, A. K., Hellhammer, D. H. and Kirschbaum, C. (1999). "Psychological and endocrine responses to psychological stress and dexamethasone/corticotrophin-releasing hormone in healthy postmenopausal women and young controls: the impact of age and a two week estradiol treatment." Neuroendocrinology **70**: 422-430.
- Kudielka, B. M., Schmidt-Reinwald, A. K., Hellhammer, D. H. and Kirschbaum, C. (2000). "Psychological stress and HPA functioning: no evidence for a reduced resilience

in healthy elderly men." Stress **3**: 229-240.

Kudielka, B. M. and Kirschbaum, C. (2003). "Awakening cortisol responses are influenced by health status and awakening time but not by menstrual cycle phase." Psychoneuroendocrinology **28**: 35-47.

Kudielka, B. M., Buske-Kirschbaum, A., Hellhammer, D. H. and Kirschbaum, C. (2004). "HPA axis response to laboratory psychosocial stress in healthy elderly adults, younger adults, and children: the impact of age and gender." Psychoneuroendocrinology **29**: 83-98.

Kudielka, B. M. and Kirschbaum, C. (2005). "Sex differences in HPA axis responses to stress: a review." Biological Psychology **69**: 113-132.

Kuhlmann, S., Kirschbaum, C. and Wolf, O. T. (2005). "Effects of oral cortisol treatment in healthy young women on memory retrieval of negative and neutral words." Neurobiology of Learning and Memory **83**: 158-162.

Kuhlmann, S. and Wolf, O. T. (2006). Cortisol effects on memory retrieval require an arousing test situation. 32nd Annual Meeting of the German Society of Psychophysiology and its Applications (DGPA): Psychology and the Brain, Dresden, Germany.

Kuhlmann, S. and Wolf, O. T. (2006). "A non-arousing test situation abolishes the impairing effects of cortisol on delayed memory retrieval in health women." Neuroscience Letters **399**: 268-272.

Kuhlmann, S. and Wolf, O. T. (2006). "Arousal and cortisol interact in modulating memory consolidation in healthy young men." Behavioural Neuroscience **120**(1): 217-223.

Kumari, M., Badrick, E., Kirschbaum, C. and Marmot, M. (2006). Sleep duration and diurnal cortisol secretion in the Whitehall II study. 37th Annual International Society for Psychoneuroendocrinology (ISPNE) Conference: Hormones and Brain from Cloning to Clinic, Leiden, The Netherlands.

Kunz-Ebrecht, S. R., Kirschbaum, C., Marmot, M. and Steptoe, A. (2004). "Differences in cortisol awakening response on work days and weekends in women and men from the Whitehall II cohort." Psychoneuroendocrinology **29**(4): 516-28.

Kushner, R. F. (1992). "Bioelectrical impedance analysis: a review of principles and applications." Journal of the American College of Nutrition **11**(2): 199-209.

Laatikainen, T. J. (1991). "Corticotrophin-releasing hormone and opioid peptides in reproduction and stress." Ann Med **23**: 489-496.

Lapidus, L., Bengtsson, C., Hallstrom, T. and Bjorntorp, P. (1989). "Obesity adipose tissue distribution and health in women. Results from a population study in Gothenburg, Sweden." Appetite (12): 25-35.

Larsson, B., Seidell, J. C. and Svardsudd, K. e. a. (1989). "Obesity, adipose tissue

- distribution and health in men. The study of men born in 1913." Appetite (13): 37-44.
- Laughlin, G. A. and Barrett-Connor, E. (2000). "Sexual dimorphism in the influence of advanced ageing on adrenal hormone levels: the Rancho Bernardo study." Clinical Endocrinology Metabolism **85**: 3561-3568.
- Laureti, S., Santeusano, F. and Falorni, A. (2001). "Recent developments on DHEA supplementation in the substitutive therapy of primary adrenal insufficiency." Expert opinion on the therapeutic patents **11**(12): 1911-1918.
- Lawrence, M. S. and Sapolsky, R. (1994). "Glucocorticoids accelerate ATP loss following metabolic insults in cultured hippocampal neurons." Brain Research **646**: 303-306.
- Lazarus, R. S. (1966). Psychological Stress and the Coping Process. New York, McGraw Hill.
- Lazarus, R. S. and Folkman, S. (1984). Stress, Appraisal and Coping. New York, Springer.
- LeBlanc, E., Janowsky, J., Chan, B. and Nelson, H. (2001). "Hormone replacement therapy and cognition: Systematic review and meta-analysis." JAMA **285**: 1489-1499.
- Lee, A. C. H., Owen, A. M., Rogers, R. D., Sahakian, B. J. and Robbins, T. W. (2000). Utility of CANTAB in functional neuroimaging. Functional neuroimaging in child psychiatry. M. a. R. Ernest, J., Cambridge University Press: 366-378.
- Leproult, R., Copinschi, G., Buxton, O. and Van Cauter, E. (1997). "Sleep loss results in an elevation of cortisol levels the next evening." Sleep **20**(10): 865-870.
- Levine, S. (1978). Cortisol changes following repeated experiences with parachute training. Psychobiology of stress. A study of coping men. H. Ursin, E. Baade and S. Levine. New York, Academic press: 51-56.
- Levine, A. S. and Billington, C. J. (1997). "Why do we eat? A neural systems approach." Annals Review of Nutrition **17**: 597-619.
- Levitt, N. S., Lambert, E. V., Woods, D., Hales, C. N., Andrew, R. and Seckl, J. R. (2000). "Impaired glucose tolerance and elevated blood pressure in low birth weight, non-obese young South African adults: early programming of cortisol axis." Clinical Endocrinology Metabolism **85**(12): 4611-4618.
- Levy, D., DeStefano, A. L., Larson, M. G., O'Donnell, C. J., Lifton, R. P., Gavras, H., Cupples, L. A. and Myers, R. H. (2000). "Evidence for a gene influencing blood pressure on chromosome 17. Genome scan linkage results for longitudinal blood pressure phenotypes in subjects from the Framingham Heart Study." Hypertension **36**: 477-483.
- Lewis, S. A. (1969). "Subjective Estimates of Sleep: An EEG Evaluation." British Journal of Psychology **60**(2): 203-8.



- Li, X. L., Aou, S., Oomura, Y., Hori, N., Fukunaga, K. and Hori, T. (2002). "Impairment of long-term potentiation and spatial memory in leptin receptor deficient rodents." Neuroscience **113**: 607-615.
- Li, G., Cherrier, M. M., Tsuang, D. W., Petrie, E. C., Colasurdo, E. A., Craft, S., Schellenberg, G. D., Peskind, E. R., Raskind, M. A. and Wilkinson, C. W. (2005). "Salivary cortisol and memory function in human aging." Neuro-Biology of Aging **In Press**.
- Lightman, S. L. (1995). "Corticotrophin-releasing factor. From stress to cognition." Nature **378**: 233-234.
- Lin, J. D., Chiou, W. K., Weng, H. F., Fang, J. T. and Liu, T. H. (2004). "Application of three-dimensional body scanner: observation of prevalence of metabolic syndrome." Clinical Nutrition **23**: 1313-1323.
- Lindheim, S. R., Legro, R. S., Bernstein, L., Stanczyk, F. Z., Vijod, M. A., Presser, S. C. and Lobo, R. A. (1992). "Behavioural stress responses in pre-menopausal and postmenopausal women and the effects of oestrogen." Am. J. Obstet. Gynecol. **167**: 1831-1836.
- Linkowski, P., Mendlewicz, J., Leclercq, R., Brasseur, M., Hubain, P., Golstein, J., Copinschi, G. and Van Cauter, E. (1985). "The 24-hour profile of adrenocorticotrophin and cortisol in major depressive illness." Journal Clinical Endocrinology Metabolism **61**: 429-438.
- Linkowski, P., Mendlewicz, J., Kerkhofs, M., Leclercq, R., Goldstein, J., Brasseur, M., Copinschi, G. and Van Cauter, E. (1987). "24-hour profiles of adrenocorticotrophin, cortisol, and growth hormone in major depressive illness: effect of antidepressant treatment." Journal Clinical Endocrinology Metabolism **65**: 141-152.
- Livingstone, D. E. W., Jones, G. C., Smith, K., Andrew, R., Kenyon, C. J. and Walker, B. (2000). "Understanding the role of glucocorticoids in obesity: tissue-specific alterations of the corticosterone metabolism in obese Zucker rats." Endocrinology **141**: 560-563.
- Ljung, T., Andersson, B. and Bengtsson, B. A. (1996). "Inhibition of cortisol secretion by dexamethasone in relation to body fat distribution: a dose response study." Obesity Research **4**: 277-282.
- Ljung, T., Holm, G., Friberg, P., Andersson, B., Bengtsson, B. A., Svensson, J., Dallman, M. F., McEwen, B. and Bjorntorp, P. (2000). "The activity of the Hypothalamic-pituitary-adrenal axis and the sympathetic nervous system in relation to Waist/Hip circumference ratio in men." Obesity Research **8(7)**: 487-495.
- Lottenberg, S. A., Giannella-Neto, D., Derendorf, H., Rocha, M., Bosco, A., Carvalho, S. V., Moretti, A. E., Lerario, A. C. and Wajchenberg, B. L. (1998). "Effect of fat distribution on the pharmacokinetics of cortisol in obesity." International Journal of Clinical Pharmacology and Therapeutics **36(9)**: 501-505.
- Lovgren, T., Hemmilla, I., Pettersson, K. and Halonen, P. (1985). Time resolved

- fluorometry in immunoassays. Alternative immunoassays. W. P. Collins. England, Wiley & Sons: 203-217.
- Luine, V., Spencer, R. L. and McEwen, B. (1993). "Effects of chronic corticosterone ingestion on spatial memory performance and hippocampal serotonergic function." Brain Research **616**: 65-70.
- Lundberg, U. (1983). "Sex differences in behaviour pattern and catecholamine and cortisol excretion in 3-6 year old day-care children." Biological Psychology **16**(1-2): 109-117.
- Lupien, S., Lecours, A. R., Lussier, I., Schwartz, G., Nair, N. P. and Meaney, M. J. (1994). "Basal cortisol levels and cognitive deficits in human aging." J. Neuroscience **14**: 2893-2903.
- Lupien, S. and Forget, H. (1995). "Glucocorticoids and cognition." Medical Science **11**: 1524-1530.
- Lupien, S., Lecours, A. R., Schwartz, G., Sharma, S., Hauger, R. L., Meaney, M. J. and Nair, N. P. (1996). "Longitudinal study of basal cortisol levels in healthy elderly subjects: evidence for subgroups." Neuro-Biology Ageing (17): 95-105.
- Lupien, S. and McEwen, B. (1997). "The acute effects of corticosteroids on cognition: integration of animal and human model studies." Brain Research **24**: 1-27.
- Lupien, S., DeLeon, M., Desanti, S., Convit, A., Tarshish, C., Nair, N. P., McEwen, B., Hauger, R. L. and Meaney, M. J. (1997). "Stress induced declarative memory impairment in healthy elderly subjects: relationship to cortisol reactivity." Journal Clinical Endocrinology Metabolism **82**: 2070-2075.
- Lupien, S. and Meaney, M. J. (1998). "Cortisol levels during human aging predict hippocampal atrophy and memory deficits." Nature Neuroscience **1**: 69-73.
- Lupien, S., Briere, S., McEwen, B., Nair, N. P. and Meaney, M. J. (1999a). "Increased cortisol levels during human aging: implication for the study of depression and dementia in later life." Neuroscience **10**: 117-140.
- Lupien, S., Gillin, J. C. and Hauger, R. L. (1999b). "Working memory is more sensitive than declarative memory to the acute effects of corticosteroids; a dose-response study." Behavioural Neuroscience **113**: 420-430.
- Lupien, S. and Lepage, M. (2001). "Stress Memory, and the hippocampus: can't live with it, can't live without it." Brain Research **127**: 137-158.
- Lupien, S., Wilkinson, C. W., Briere, S., Menard, C., Ng, Y. K. and Nair, N. P. (2002a). "The modulatory effects of corticosteroids on cognition: studies in young human populations." Psychoneuroendocrinology **27**: 401-416.
- Lupien, S., Wilkinson, C. W., Briere, S., NG, Y. K., Meaney, M. J. and Nair, N. P. (2002b). "Acute modulation of aged human memory by pharmacological manipulation of glucocorticoids." Journal Clinical Endocrinology & Metabolism **87**: 3798-3807.

- Lupien, S., Fiocco, A., Wan, N., Maheu, F. S., Lord, C., Schramek, T. and Thanh Tu, M. (2005). "Stress hormones and human memory function across the lifespan." Psychoneuroendocrinology **30**: 225-242.
- Luria, A. R. (1966). Higher cortical functions in man. New York.
- Maes, M., Calabrese, J. and Meltzer, H. Y. (1994). "Effects of Age on Spontaneous cortisolemia of normal volunteers and depressed patients." Psychoneuroendocrinology **19**: 79-84.
- Maes, H. H., Neale, M. C. and Eaves, L. J. (1997). "Genetic and environmental factors in relative body weight and human adiposity." Behaviour Genetics **27**: 325-351.
- Maheu, F. S., Collicutt, P., Kornik, R., Moszkowski, R. and Lupien, S. (2005). "The perfect time to be stressed: A differential modulation of human memory by stress applied in the morning or in the afternoon." Progress in Neuro-Psychopharmacology & Biological psychiatry. **29**: 1281-1288.
- Manuck, S. B., Cohen, S., Rabin, B. S. and Muldoon, M. F. (1991). "Individual differences in cellular immune response to stress." Psychological Science **2**: 111-115.
- Marin, P., Darin, N., Amemiya, T., Andersson, B., Jern, S. and Bjorntorp, P. (1992). "Cortisol Secretion In Relation To Body Fat Distribution in Obese Pre-menopausal Women." Metabolism **41(8)**: 882-886.
- Marsland, A. L., Manuck, S. B., Wood, P. J., Rabin, B. S., Muldoon, M. F. and Cohen, S. (1995). "Beta2-adrenergic receptor density and cardiovascular response to mental stress." Physiological Behaviour **57**: 1163-1167.
- Mason, J. W. (1968). "A review of psychoendocrine research on the pituitary-adrenal cortical system." Psychosomatic Medicine(30).
- Matthews, D. F., Hosker, J. P., Naylor, B. A., Treacher, D. F. and Turner, R. C. (1985). "Homeostasis model assessment: insulin resistance and Beta cell function from fasting plasma glucose and insulin concentration in man." Diabetologia **28**: 412-419.
- McEwen, B., de Kloet, E. R. and Rostene, W. (1986). "Adrenal steroid receptors and action in the nervous system." Physiological Review **66**: 1121-1188.
- McEwen, B. (1995). "Stress and the brain: a paradoxical role for adrenal steroids." Vitamins and Hormones **Litwack, G.D. ed**: 371-402.
- McEwen, B. (1997). "Possible mechanisms for atrophy of the human hippocampus." Mol Psychiatry **2**: 255-262.
- McEwen, B. S. (1998). "Protective and damaging effects of stress mediators." New England journal of medicine **338**: 171-179.
- McEwen, B. and Wingfield, J. C. (2003). "The concept of allostasis, in biology and biomedicine." Hormones and Behaviour **43**: 2-15.

- Meeran, K., Hattersley, A., Mould, G. and Bloom, S. R. (1993). "Venepuncture causes rapid rise in plasma ACTH." British Journal Clinical Practice **47**: 246-247.
- Melamed, S., Kushnir, T. and Shirom, A. (1992). "Burnout and risk factors for cardiovascular diseases." Behavioural Medicine **18**: 53-60.
- Melamed, S., Ugartern, U., Shirom, A., Kahana, L., Lerman, Y. and Froom, P. (1999). "Chronic burnout, somatic arousal and elevated salivary cortisol levels." Journal of Psychosomatic Research **46**(6): 591-598.
- Mellitus, D. (1997). "Report of the expert committee on the diagnosis and classification of diabetes mellitus." Diabetes Care **20**: 1183-1187.
- Messier, C., Tsiakas, M., Gagnon, M., Desrochers, A. and Awad, N. (2003). "Effect of age and gluco-regulation on cognitive performance." Neurobiology of Aging **24**: 985-1003.
- Messier, C. (2005). "Impact of impaired glucose tolerance and type 2 diabetes on cognitive aging." Neurobiology of Aging **26S**: S26-S30.
- Migeon, C. J., Green, O. C. and Eckert, J. P. (1963). "Study of Adrenocortical Function in Obesity." Metabolism **12**: 718.
- Miller, S. M. and Kirsch, N. (1987). Sex differences in cognitive coping with stress. Gender and Stress. R. S. Barnett, L. Biener and G. K. Baruch. New York, Free Press: 278-307.
- Miller, W. L. and Tyrell, J. B. (1995). Endocrinology and Metabolism. P.Fehlig, D. Baxter and L. A. Frohman. New York, McGraw Hill Inc: 555-711.
- Moe, K. E. (1999). "Reproductive hormones, aging and sleep." Endocrinology Review **17**: 339-348.
- Moe, K. E., Larsen, L. H., Vitello, M. V. and Prinz, P. N. (2001). "Oestrogen replacement therapy moderates the sleep description associated with nocturnal blood sampling." Sleep **24**(8): 886-894.
- Moffat, S. D. (2002). "Longitudinal assessment of serum free testosterone concentration predicts memory performance and cognitive status in elderly men." Journal Clinical Endocrinology & Metabolism **87**: 5001-5007.
- Molarius, A., Seidell, J. C., Sans, S., Tuomilehto, J. and Kuulasmaa, K. (1999). "Waist and hip circumferences, and waist hip ratio in 19 populations of the WHO MONICA project." International Journal Obesity Related Metabolic Disorders **23**: 116-25.
- Mommersteeg, P. M. C., Heijnen, C., Verbraak, M. J. P. M. and van Doornen, L. J. (2006). "Clinical burnout is not reflected in the cortisol awakening response, the day-curve or the response to a low-dose dexamethasone suppression test." Psychoneuroendocrinology **31**: 216-225.

- Morgan, C. A., Cho, T., Hazlett, G., Coric, V. and Morgan, J. (2002). "The impact of burnout on human physiology and on operational performance: a prospective study of soldiers enrolled in the combat diver qualification course." Journal Biological Medicine 75: 199-205.
- Morineau, G., Boudi, A., Barka, A., Gourmelen, M., Degeilh, F., Hardy, N., Al-Halnak, A., Soliman, H., Gosling, J., Julien, R., Boudou, P. and Aubert, P. (1997). "Radioimmunoassay of cortisone in serum, urine, and saliva to assess the status of the cortisol-cortisone shuttle." Clinical Chemistry 43(8): 1397-1407.
- Mortola, J. F., Liu, J. H., Gillin, J. C., Rasnaussen, D. D. and Yen, S. S. (1967). "Pulsatile rhythms of adrenocorticotrophin (ACTH) and cortisol in women with endogenous depression evidence of increased ACTH pulse frequency." J. Clin Endocrinol Metab 65: 962-68.
- Moyer, A., Rodin, J., Grilo, C., Cummings, N., Larson, L. and Rebuffe-Scriffe, M. (1994). "Stress-induced cortisol response and fat distribution in women." Obesity Research 2: 255-62.
- Munck, A., Guyre, P. M. and Holbrook, N. J. (1984). "Physiological functions of glucocorticoids in stress and their relation to pharmacological actions." Endocrinology Review 5: 25-44.
- Natelson, B. H., Ottenweiler, J. E. and Cook, J. A. (1988). "Effect of stressor intensity on habituation of the adrenocortical stress response." Physiology Behaviour 43: 41-46.
- National institutes of Health and National Heart, L. a. B. i. (1998). "Clinical guidelines on the identification, evaluation and treatment of overweight and obesity in adults." US department of Health and Human services, Public health services.
- NCEP (2001). Executive summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert panel on detection, evaluation and treatment of High Blood Cholesterol In Adults, JAMA: 2486-2497.
- Neel, J. V. (1962). "Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"." American Journal of Human Genetics 14: 353-362.
- Nelson, J. (1982). "The National Adult Reading Test (NART): test manual." NFER - Nelson.
- Newcomer, J. W., Craft, S., Hershey, T., Askins, K. and Bardgett, M. E. (1994). "Glucocorticoid-induced impairment in declarative memory performance in adult humans." J. Neuroscience 14: 2047-2053.
- Newcomer, J. W., Selke, G., Kelly, A. K., Paras, L. and Craft, S. (1995). "Age-related differences in glucocorticoid effect on memory in human subjects." Social Neuroscience Abstract 21: 161.
- Newcomer, J. W., Selke, G., Meslon, A. K., Heershey, T., Craft, S., Richards, K. and Alderson, A. L. (1999). "Decreased memory performance in healthy humans induced by stress-level cortisol." Archives of General Psychiatry 56: 527-533.

- NHMHRDP, N. H. M. H. R. D. P. (1999). "Native Hawaiian Mental Health Research Development Program (NHMHRDP): Program summary and database variable codes."
- Nicholson, A. N., Stone, B. M., Clarke, C. H. and Ferres, H. M. (1976). "Effect of N-demethyl diazepam (nordiazepam) and precursor, potassium clorazepate, on sleep in man." British Journal Clinical Pharmacology **3**: 429-438.
- Nicolson, N., Storms, C., Ponds, R. and Sulon, J. (1997). "Salivary cortisol levels and stress reactivity in human ageing." Gerontol. A Biol. Sci. Med. Sci **52**: M68-M75.
- Nolten, W. E., Lindheimer, M. D., Rueckert, S. O. and Erhlich, E. N. (1980). "Diurnal patterns and regulation of cortisol secretion in pregnancy." Journal Clinical Endocrinology & Metabolism **65**: 466-472.
- O'Carroll, R., Walker, M., Dunan, J., Murray, C., Blackwood, D., Ebmeier, K. P. and Goodwin, G. M. (1992). "Selecting controls for schizophrenia research studies: the use of the national adult reading test (NART) is a measure of pre-morbid ability." Schizophrenia Research **8**: 137-141.
- O'Connell, M., Danforth, E. and Horton, E. S. (1973). "Experimental obesity in Man. III. Adrenocortical function." Journal Clinical Endocrinology & Metabolism **36**: 323.
- O'Connor, P. J. and Corrigan, D. L. (1987). "Influence of short-term cycling on salivary cortisol levels." Medicine and Science in Sports and Exercise **19**: 224-228.
- Obeyon, M. M. (2002). "Epidemiology of insomnia: what we know and what we still need to know." Sleep **6**(2): 97-111.
- Ockenfels, M. C., Smyth, J., Porter, L. S., Kirschbaum, C., Hellhammer, D. and Stone, D. (1995). "The effect of Daily hassles on salivary cortisol." Verhaltenstherapie **5**(1): 16-20.
- Otte, C., Hart, S., Neylan, T. C., Marmar, C. R., Yaffe, K. and Mohr, D. C. (2005). "A meta-analysis of cortisol response to challenge in human aging: importance of gender." Psychoneuroendocrinology **30**: 80-91.
- Owen, A. M., Iddon, J. L., J.R., H., Summers, B. A. and Robbins, T. W. (1997). "Spatial and Non Spatial working memory at different stages of Parkinson's disease." Neuropsychologia **35**: 519-532.
- Parrott, A. C. and Hindmarch, I. (1978). "Factor analysis of a sleep evaluation questionnaire." Psychology Medicine **8**: 325-329.
- Pasquali, R. and Vincennati, V. (2000). "Activity of the hypothalamic - pituitary - adrenal axis in different obesity phenotypes." International Journal of Obesity **24**(2): s47-s49.
- Pasquali, R., Ambrosi, B., Armanini, D., Cavagnini, F., Uberti, E. D. and Del Rio, G. e. a. (2002). "Cortisol and ACTH response to oral dexamethasone in obesity and effects of sex, body fat distribution, and dexamethasone concentrations: a dose-response study."

Journal Clinical Endocrinology & Metabolism **87**: 166-175.

Pavlidis, C., Watanabe, Y. and McEwen, B. (1993). "Effects of glucocorticoids on hippocampal long term potentiation." Neuroreport **3**: 183-192.

Pavlidis, C., Kimura, A., Magarinos, A. M. and McEwen, B. (1994). "Type 1 adrenal steroid receptors prolong hippocampal long term potentiation." Neuroreport **5**: 2673-2677.

Pavlidis, C., Kimura, A., Mararinos, A. M. and McEwen, B. (1995). "Hippocampal homo-synaptic long-term depression/Depotentialion induced by adrenal steroids." Neuroscience in press.

Pavlidis, C., Watanabe, Y., Magarinos, A. M. and McEwen, B. (1995). "Opposing role of Adrenal steroid type 1 and type 11 receptors on hippocampal long term potentiation." Neuroscience **68**: 387-394.

Pedersen, S., Jonler, M. and Richelsen, B. (1994). "Characterization of regional and gender differences in glucocorticoid receptors and lipoprotein lipase activity in human adipose tissue." Journal Clinical Endocrinology & Metabolism **78**: 1354-9.

Petrovsky, N. and Harrison, L. C. (1995). "Cytokine based human whole blood assay for the detection of antigen-reactive T cells." Journal Immunology **186**: 37-46.

Petrovsky, N. and Harrison, L. C. (1997). "Diurnal rhythmicity of human cytokine production: a dynamic disequilibrium in T helper cell type 1/T helper cell type 2 balance?" Journal of Immunology **158**: 5163-5168.

Phillips, D. I. W., Barker, D. J., Fall, C. H., Seckl, J. R., Whorwood, C. B., Wood, P. J. and Walker, B. (1998). "Elevated plasma cortisol concentrations: a link between low birth weight and the insulin resistance syndrome?" J. Clin Endocrinol Metab **83**: 757-760.

Phillips, D. I. W., Walker, B., Reynolds, R. M., Flanagan, D. E., Wood, P. J., Osmond, C., Barker, D. J. and Whorwood, C. B. (2000). "Low birth weight predicts elevated plasma cortisol concentrations in adults from 3 populations." Hypertension **35**: 1301-1306.

Pickup, J. C. and Crook, M. A. (1998). "Is type II diabetes mellitus a disease of the innate immune system?" Diabetologia **41**: 1241-1248.

Pitman, D. L., Ottenweiler, J. E. and Natelson, B. H. (1988). "Plasma corticosterone levels during repeated presentation of two intensities of restraint stress: Chronic stress and habituation." Physiological Behaviour **43**: 47-55.

Pitman, D. L., Ottenweiler, J. E. and Natelson, B. H. (1990). "Effect of stressor intensity on habituation and sensitization of glucocorticoid response in rats." Behavioural Neuroscience **104**: 28-36.

Port, K. (1991). "Serum and saliva cortisol responses and blood lactate accumulation during incremental exercise testing." International Journal of Sports Medicine **12**: 490-

494.

Porter, R. J., Barnett, N. N., Idely, A., McGuckin, E. A. and O'Brien, T. O. (2002). "Effects of hydrocortisone administration on cognitive function." J. Psychopharmacology **16**: 65-71.

Prentice, A. M. and Jebb, S. A. (2001). "Beyond Body mass index." Obesity Research **2**: 141-147.

Prettyman, R. J., Cordle, C. J. and Cook, G. D. (1993). "A three-month follow-up of psychological morbidity after early miscarriage." British Journal Medical Psychology **66**: 363-372.

Prinz, P. N., Bailey, S. L. and Woods, D. L. (2000). "Sleep impairments in healthy Seniors: Roles of stress, cortisol, and interleukin-1 beta." Chronobiology International **17**(3): 391-404.

Probst, M., Bulbulian, R. and Knapp, C. (1997). "Haemodynamic responses to the stroop and cold pressor tests after sub-maximal cycling exercise in normative males." Physiology Behaviour **62**: 1283-1290.

Prüssner, J. C., Wolf, O. T., Hellhammer, D. H., Buske-Kirschbaum, A., von Auer, K., Jobst, S., Kaspers, F. and Kirschbaum, C. (1997). "Free cortisol levels after awakening: A reliable biological marker for assessment of adrenocortical activity." Life Sciences **61**: 2539-2549.

Prüssner, J. C., Hellhammer, D. H. and Kirschbaum, C. (1999). "Burnout, perceived stress and cortisol response to awakening in teachers." Psychosomatic Medicine **61**: 197-204.

Prüssner, M., Hellhammer, D. H., Prüssner, J. C. and Lupien, S. J. (2003). "Self-reported depressive symptoms and stress levels in healthy young men: associations with cortisol response to awakening." Psychosomatic Medicine **65**: 92-99.

Putignano, P., Dubini, A., Toja, P., Invitti, C., Bonfanti, S., Redaelli, G., Zappulli, D. and Cavagnini, F. (2001). "Salivary cortisol measurement in normal-weight, obese and anorexic women: comparison with plasma cortisol." European journal of Endocrinology **145**: 165-171.

Putman, P., van Honk, J., Kessels, R. P. C., Mulder, M. and Koppeschaar, H. P. F. (2004). "Salivary cortisol and short and long-term memory for emotional faces in healthy young women." Psychoneuroendocrinology **29**: 953-960.

Rabbit, P. and Lowe, C. (2000). "Patterns of Cognitive ageing." Psychological Research **63**(3-4): 308-316.

Raff, H., Raff, J. L., Duthie, E. H., Wilson, C. R., Sasse, E. A., Rudman, I. and Mattson, D. (1999). "Elevated salivary cortisol in the evening in healthy elderly men and women: correlation with bone mineral density." Gerontol. A Biol. Sci. Med. Sci **54**: M479-M483.



- Rahman, S., Robbins, T. W. and Sahakian, B. J. (1999). "Comparative cognitive neuropsychological studies of frontal lobe function: Implications for therapeutic strategies in frontal variant frontotemporal dementia." Dementing Geriatric Cognitive Disorders 10(Suppl. I): 15-28.
- Raja, C., Hansen, R., Baber, R. and Allen, B. (2004). "Hip girth as a predictor of abdominal adiposity in postmenopausal women." Nutrition 20: 772-777.
- Rask, E., Walker, B., Sonderberg, S., Livingstone, D. E. W., Eliasson, M., Johnson, O., Andrew, R. and Olsson, T. (2002). "Tissue-specific changes in peripheral cortisol metabolism in obese women: Increased adipose 11 $\beta$ -Hydroxysteroid dehydrogenase type 1 Activity." The Journal of Clinical Endocrinology & Metabolism 87(7): 3330-3336.
- Reaven, G. M. (1988). "Role of Insulin resistance in human disease." Diabetes Care 37: 1595-1607.
- Rebuffe-Scriffe, M., Walsh, U. A., McEwen, B. and Rodin, J. (1991). "Effect of chronic stress and exogenous glucocorticoids on regional fat distribution and metabolism." Physiological Behaviour 52: 583-590.
- Reilly, C. E. (2000). "Gender-Specific brain activation during visuospatial navigation." Journal Neurology 247: 580-581.
- Reul, J. M. and de Kloet, E. R. (1985). "Two Receptor Systems for Corticosterone in the rat brain: microdistribution and differential occupation." Endocrinology 117(6): 2505-11.
- Rey, A. (1964). L'Examen Clinique en Psychologie. Paris, Press Universitaire de France.
- Reynolds, R. M., Walker, B., Phillips, D. I. W., Sydall, H. E., Andrew, R., Wood, P. J. and Whorwood, C. B. (2001). "Altered control of cortisol secretion in adult men with low birth weight and cardiovascular risk factors/." Journal Clinical Endocrinology Metabolism 86: 245-250.
- Rivera, M. P. and Svec, F. (1989). "Is Cortisol involved in upper-body obesity." Medical Hypotheses 30: 95-100.
- Roberts, A. D. L., Wessley, T., Chalder, T., Papadopoulos, A. and Cleare, A. J. (2004). "Salivary Cortisol response to awakening in chronic fatigue syndrome." British Journal Psychiatry 184: 136-141.
- Rockall, A. G., Sohaib, S. A., Evans, D., Kaltsas, G., Isidori, A. M., Monson, J. P., G.M., B., Grossman, A. B. and Reznick, R. H. (2003). "Computed tomography assessment of fat distribution in male and female patients with Cushing's syndrome." European journal of Endocrinology 149: 561-567.
- Rohrman, S., Hennig, J. and Netter, P. (1999). "Changing psychobiological stress reactions by manipulating cognitive processes." International Journal Psychophysiology 33: 149-161.

- Roozendaal, B. and McGaugh, J. L. (1997). "Basolateral amygdala lesions block the memory-enhancing effect of glucocorticoid administration in the dorsal hippocampus of rats." Eur J. Neurosci. **9**: 76-83.
- Roozendaal, B., Nguyen, B. T., Power, A. E. and McGaugh, J. L. (1999). "Basolateral amygdala noradrenergic influence enables enhancement of memory consolidation induced by hippocampal glucocorticoid receptor activation." Proceedings of National Academy of Sciences **96**: 11642-11647.
- Roozendaal, B. (2000). "Glucocorticoids and the regulation of memory consolidation." Psychoneuroendocrinology **25**: 213-238.
- Rose, R. M. (1980). "Endocrine responses to stressful psychological events. Advances in Psychoneuroendocrinology." Psychiatric Clinical of North America **3**: 251-276.
- Rosmond, R., Dallman, M. F. and Bjorntorp, P. (1998). "Stress-related cortisol secretion in men: relationships with abdominal obesity and endocrine, metabolic and haemodynamic abnormalities." Journal Clinical Endocrinology & Metabolism **83**: 1853-1859.
- Rosmond, R. and Bjorntorp, P. (1999b). "Psychosocial and socio-economic factors in women and their relationship to obesity and regional fat distribution." International Journal Obesity Related Metabolic Disorders **23**: 138-145.
- Rosmond, R., Chagnon, Y. C., Holm, G., Chagnon, M., Perusse, L., Lindell, K., Carlsson, B., Bouchard, C. and Bjorntorp, P. (2000). "A glucocorticoid receptor gene marker is associated with abdominal obesity, leptin, and dysregulation of the hypothalamic-pituitary-adrenal axis." Obesity Research **8**(3): 211-218.
- Rosmond, R., Holm, G. and Bjorntorp, P. (2000). "Food induced cortisol secretion in relation to anthropometric, metabolic and haemodynamic variables in men." International Journal Obesity Related Metabolic Disorders **24**(4): 416-22.
- Rosmond, R. and Bjorntorp, P. (2001). "Alterations in the hypothalamic-pituitary-adrenal axis in metabolic syndrome." Endocrinologist **11**(6): 491-497.
- Rush, E. C., Plank, L. D. and Lualaba, M. S. (1997). "Prediction of Percentage Body Fat from Anthropometric measurements: comparison of New Zealand, European and Polynesian young women." American Journal of Clinical Nutrition **66**: 2.
- Ryan, C. M. (2003). "Hormones, genetic factors and gender differences in cardiovascular disease." Cardiovascular Research **53**: 550-7.
- Ryan, C. M. (2005). "Diabetes, aging, and cognitive decline." Neurobiology of Aging **26S**: S21-S25.
- Salkovskis, P. M., Storer, D., Atha, C. and Warwick, H. M. (1990). "Psychiatric morbidity in an accident and emergency department. Characteristics of patients at presentation and one month follow-up." British Journal of Psychiatry **156**: 483-487.

- Sandeep, T., Yau, J. L. W., MacLulich, A. M. J., Noble, J., Deary, I. J., Walker, B. and Seckl, J. R. (2004). "11 $\beta$  - Hydroxysteroid Dehydrogenase inhibition improves cognitive function in healthy elderly men and type 2 diabetics." PNAS **101**(17): 6734-6739.
- Sapolsky, R., Krey, L. and McEwen, B. (1986). "The Neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis." Endocrinology Review **7**: 284-301.
- Sapsee, A. T. (1997). "High cortisol diseases and anti-cortisol therapy." Psychoneuroendocrinology **22**: S3-10.
- Scheen, A. J., Byrne, M. M., Plat, L., Leproult, R. and Van Cauter, E. (1996). "Relationships between sleep quality and glucose regulation in normal humans." American Journal of Physiology **271**(34): E261-E270.
- Schlottz, W., Hellhammer, J., Schulz, P. and Stone, A. A. (2004). "Perceived work overload and chronic worrying predict weekend-weekday differences in the cortisol awakening response." Psychosomatic Medicine **66**(2): 207-214.
- Schmidt, L. A., Fox, N. A., Goldberg, M. C., Smith, C. C. and Schulkin, J. (1999). "Effects of acute prednisone administration on memory, attention and emotion in healthy human adults." Psychoneuroendocrinology **24**: 461-483.
- Schmidt-Reinwald, A., Prüssner, J. C., Hellhammer, D. H., Federenko, I., Rohleder, N., Schumeyer, T. and Kirschbaum, C. (1999). "The cortisol response to awakening in relation to pharmacological and psychological challenge tests, and a 12 hour circadian cortisol rhythm." Life Sciences **64**: 1683-1660.
- Schommer, N. C., Hellhammer, D. H. and Kirschbaum, C. (2003). "Dissociation between reactivity of the HPA-axis and the sympathetic adrenal medullary system to repeated psychosocial stress." Psychosomatic Medicine **65**: 450-460.
- Schulz, P., Kirschbaum, C., Prüssner, J. C. and Hellhammer, D. H. (1998). "Increased free cortisol secretion after awakening in chronically stressed individuals due to work overload." Stress Medicine **14**: 91-97.
- Seeman, T. E., Singer, B. and Charpentier, P. (1995). "Gender differences in patterns of HPA axis response to challenge: MacArthur studies of successful aging." Psychoneuroendocrinology **20**: 711-725.
- Seeman, T. E., McEwen, B., Singer, B., Albert, M. S. and Rowe, J. W. (1997). "Increase in Urinary cortisol excretion and memory declines: MacArthur studies of successful aging." Journal Clinical Endocrinology Metabolism **257**: 537-539.
- Segal, K. R., Dunaif, A. and Gutin, B. (1986). "Body composition not body weight is related to cardiovascular disease risk factors and sex hormones levels in men." Journal of Clinical Investigation **80**: 1050.
- Seifritz, E., Hemmeter, U., Trachsel, L., Lauer, C. J., Hatzinger, M., Emrich, H. M., Holsboer, F. and Holsboer-Trachslers, E. (1995). "Effects of flumazenil on recovery sleep and hormonal secretion after sleep deprivation in male controls."

Psychopharmacology **120**: 449-456.

Selye, H. (1956). The Stress of Life. New York, McGraw-Hill.

Selye, H. (1974). Stress without distress. J. B. Lippincott. New York.

Selye, H. (1979). The stress concept and some of its implications. Human stress and cognition: An information processing result. V. H. D. Warburton. New York, Wiley & sons.

Sephton, S., Sapolsky, R., Kraemer, H. and Spiegel, D. (2000). "Diurnal Cortisol rhythm as a predictor of breast cancer survival." National Cancer Institute **92**: 994-1000.

Sher, L. (2004). "Daily hassles, cortisol, and the pathogenesis of depression." Medical Hypotheses **62**: 198-202.

Sherwin, B. B. (1997). "Oestrogen effects cognition in menopausal women." Neurology **48**: S21-S26.

Shirom, A. (1989). Burnout in work organisations. International review of industrial and organisational psychology. C. L. Cooper. New York, John Wiley & Sons.

Shirom, A., Westman, M., Shamai, O. and Carel, R. S. (1997). "The effects of work overload and burnout on cholesterol and triglyceride levels: the moderating effects of emotional reactivity among male and female employees." Occupational Health Psychology **2**: 275-288.

Simkin, B. (1961). "Urinary 17-Ketosteroid and 17-Ketogenic steroid excretion in patients." New England journal of medicine **264**: 974-977.

Singh-Manoux, A. and Marmot, M. (2005). "High blood pressure was associated with cognitive function in middle-age in the Whitehall II study." Journal of Clinical Epidemiology **58**: 1308-1315.

Skosnik, P. D., Chatterton Jr, R. T., Swisher, T. and Park, S. (2000). "Modulation of attentional inhibition by norepinephrine and cortisol after psychological stress." International Journal of Psychophysiology **36**: 59-68.

Smeets, T., Jelicic, M. and Merckelbach, H. (2005). "The effect of acute stress on memory depends on word valence." International Journal of Psychophysiology **In Press**.

Smeets, T., Jelicic, M. and Merckelbach, H. (2006). "Stress-induced cortisol responses, sex differences, and false recollections in a DRM paradigm." Biological Psychology **72(2)**: 164-172.

Smyth, J. M., Ockenfels, M. C., Gorin, A. A., Catley, D., Porter, L. S., Kirschbaum, C., Hellhammer, D. H. and Stone, A. A. (1997). "Individual differences in the diurnal cycle of cortisol." Psychoneuroendocrinology **22**: 89-105.

Snaith, R. P. and Zigmond, A. S. (1994). "HADS: Hospital Anxiety and Depression

Scale." Windsor.

Snieder, H., van Doornen, L. J. and Boomsma, D. I. (1999). "Dissecting the genetic architecture of lipids, lipoproteins, and apolipoproteins: lessons from twin studies." Arteriosclerosis Thrombosis and Vascular Biology **19**: 2826-2834.

Soini, E. and Kojola, H. (1983). "Time resolved fluorometer for lanthanide chelates - a new generation of nonisotopic immunoassays." Clinical Chemistry **29**: 65-68.

Sorensen, T. I., Sonne-Holm, S., Christensen, U. and Kreiner, S. (1982). "Reduced intellectual performance in extreme overweight." Human Biology **54**: 765-775.

Spath-Schwalbe, E., Gofferje, M., Kern, W., Born, J. and Fehm, H. L. (1991). "Sleep disruption alters nocturnal ACTH and cortisol patterns." Biological Psychiatry **29**: 575-584.

Spath-Schwalbe, E., Scholler, T., Kern, W., Fehm, H. L. and Born, J. (1992). "Nocturnal Adrenocorticotrophin and cortisol secretion depends on sleep duration and decreases in association with spontaneous awakening in the morning." Clinical Endocrinology & Metabolism **81**: 1431-1435.

Spencer, R. L., Miller, A. H., Moday, H., Stein, M. and McEwen, B. (1993). "Diurnal differences in basal and acute stress levels of type I and type II adrenal steroid activation in neural and immune tissues." Endocrinology **133**: 1941-1950.

Spielberger, C. D., Gorsuch, R. L. and Lushene, R. E. (1970). State-Trait Anxiety Inventory manual. California, Consulting Psychologists Press, Inc.

Spielberger, C. D. (1979). Preliminary manual for the State-Trait Personality Inventory (STPI), University of South Florida.

Spielberger, C. D. and Diaz-Guerrero, R. (1983). Cross-cultural anxiety, Vol 2. Washington DC, Hemisphere/Wiley.

Stanford, S. C. (1993). Monoamines in response and adaptation to stress. Stress: from synapse to syndrome. S. P. Stanford SC. New York, Academic Press: 281-331.

Stanford, S. C. and Salmon, P. (1993). Stress: From Synapse to Syndrome. London, Academic Press.

Starkman, M. N., Scheingart, D. E. and Schork, A. (1986). "Cushing's syndrome after treatment: changes in cortisol and ACTH levels, and amelioration of the depressive syndrome." Psychiatry Research **19**: 177-188.

Starkman, M. N., Gebarski, S. S., Berent, S. and Stcheingart, D. E. (1992). "Hippocampal formation volume, memory dysfunction and cortisol levels in patients with Cushing's syndrome." Biology Review **32**: 756-765.

Starkman, M. N., Giordani, B., Gebarski, S. S., Berent, S., Schork, A. and Scheingart, D. E. (1999). "Decrease in cortisol reverses human hippocampal atrophy following treatment of Cushing's disease." Biology Psychiatry **46**: 1595-1602.

- Starkman, M. N., Giordani, B., Gebarski, S. S. and Schteingart, D. E. (2003). "Improvement in learning associated with increase in hippocampal formation volume." 53 3(233-238).
- Stephoe, A., Kunz-Ebrecht, S. R., Brydon, L. and Wardle, J. (2004). "Central adiposity and cortisol responses to waking in middle-aged men and women." International Journal of Obesity 28: 1168-1173.
- Stewart, P. M. (1996). "11 $\beta$  - Hydroxysteroid Dehydrogenase - implications for clinical medicine." Clinical Endocrinology Metabolism 44: 493-99.
- Stewart, P. M., Krozowski, Z. S. and Gupta, A. (1996). "Hypertension in the syndrome of apparent mineralcorticoid excess due to mutation of the type 2 11 $\beta$ -hydroxysteroid dehydrogenase gene." Lancet 347: 88-91.
- Stice, E., Fisher, M. and Martinez, E. (2004). "Eating disorder diagnostic scale: Additional evidence of reliability and validity." Psychological Assessment 16(1): 60-71.
- Stone, A. A., Schwartz, J. E., Smyth, J., Kirschbaum, C., Cohen, S., Hellhammer, D. and Grossman, S. (2001). "Individual differences in the diurnal cycle of salivary free cortisol: a replication of flattened cycles for some individuals." Psychoneuroendocrinology 26: 295-303.
- Strachan, M. W., Deary, I. J., Ewing, F. M. and Frier, B. M. (1997). "Is type II diabetes associated with increased risk of cognitive dysfunction? A critical review of published studies." Diabetes Care 20: 438-445.
- Strain, G. W., Zumoff, B., Strain, J. J., Levin, J. and Fukushima, D. K. (1980). "Cortisol production in obesity." Metabolism 29(980-985).
- Streeten, D. H. P., Stevenson, C. T. and Dalakos, T. G. (1969). "Diagnosis of hypercortisolism: biochemical criteria differentiating patients from lean and obese normal subjects and from females on oral contraceptives." J. Clin Endocrinol Metab 29: 1191.
- Sutherland, S. (1995). Macmillan Dictionary of Psychology. London, The Macmillan press Ltd.
- Szenas, P. and Pattee, C. J. (1959). "Studies of adrenocortical function in obesity." Journal Clinical Endocrinology & Metabolism 19: 344-348.
- Szostak-Wegierek, D., Bjorntorp, P., Marin, P., Lindstedt, G. and Anderson, B. (1996). "Influence of smoking on hormone secretion in obese and lean female smokers." Obesity Research 4: 321-8.
- Takahashi, T., Ikeda, K., Ishikawa, M., Tsukasaki, T., Nakama, D., Tanida, S. and Kameda, T. (2004). "Social stress-induced cortisol elevation acutely impairs social memory in humans." Neuroscience Letters 363: 125-130.
- Tannin, G. M., Agarwal, A. K., Monder, C., New, M. I. and White, P. C. (1991). "The

- human gene for 11 $\beta$  - Hydroxysteroid Dehydrogenase: structure, tissue distribution and chromosomal localization." Journal Biological Chemistry **266**: 16653-58.
- Tarrasch, R., Laudon, M. and Zisapel, N. (2003). "Cross-cultural validation of the Leeds Sleep evaluation questionnaire (LSEQ) in insomnia patients." Human Psychopharmacology **18**: 603-610.
- Tataranni, P. A., Larson, D. E., Snitker, S., Young, J. B., Flatt, J. P. and Ravussin, E. (1996). "Effects of glucocorticoids on energy metabolism and food intake in humans." American Journal Physiology **34**: E317-325.
- Tchernof, A., Nolan, A., Sites, C. K., Ades, P. A. and Poehlman, E. T. (2002). "Weight loss reduces C-Reactive protein levels in obese postmenopausal women." Circulation **105**: 564-569.
- Tempel, D. L. and Leibowiz, S. F. (1994). "Adrenal steroid receptors: interactions with brain neuropeptide systems in relation to nutrient intake and metabolism." J. Neuroendocrinology **6**: 479-501.
- Terrazzino, S., Perego, C. and De Simoni, M. G. (1995). "Effect of development of habituation to restraint stress on hypothalamic noradrenaline release and adrenocorticotrophin secretion." Neurochemistry **65**: 263-267.
- Thorn, L., Hucklebridge, F., Esgate, A., Evans, P. and Clow, A. (2004). "The effect of dawn simulation on the cortisol response to awakening in healthy participants." Psychoneuroendocrinology **29**: 925-930.
- Thorn, E., Hucklebridge, F., Evans, P. and Clow, A. (2006). "Suspected non-adherence and weekend versus week day differences in the awakening cortisol response." Psychoneuroendocrinology **31**: 1009-1018.
- Tombaugh, G. and Sapolsky, R. (1992). "Corticosterone accelerates hypoxia induced ATP loss in cultured hippocampal astrocytes." Brain Research **588**: 154-159.
- Traustadottir, T., Bosch, P. R. and Matt, K. S. (2005). "The HPA axis response to stress in women: effects of aging and fitness." Psychoneuroendocrinology **30**: 392-402.
- Troisi, A. (2001). "Gender differences in vulnerability to social stress: a Darwinian perspective." Psychological Behaviour **73**: 443-449.
- Tunn, S., Mollmann, H., Barth, J., Derendorf, H. and Krieg, M. (1992). "Simultaneous measurement of cortisol in serum and saliva after different forms of cortisol administration." Clinical Chemistry **38**: 1491-1494.
- Uhart, M., Chong, R. Y., Oswald, L., Lin, P. and Wand, G. S. (2006). "Gender differences in hypothalamic-pituitary adrenal (HPA) axis reactivity." Psychoneuroendocrinology **31**: 642-652.
- Umeda, T., Hiramatsu, R., Iwaoka, T., Shimada, T., Miura, F. and Sato, T. (1981). "Use of saliva for monitoring unbound free cortisol levels in serum." Clinical Chimica Acta **110**: 245-253.

- Vagg, P. R. and Spielberger, C. D. (1980). "Is the State-Trait anxiety inventory multidimensional?" Personal and Individual differences 1: 207-214.
- Vamvakopoulos, N. C. and Chrousos, G. P. (1993). "Evidence of direct estrogenic regulation of human corticotrophin-releasing hormone gene expression. Potential implications for the sexual dimorphism of the stress response and immune/inflammatory reaction." Journal of Clinical Investigation 92: 1896-1902.
- Van Cauter, E. and Turek, F. W. (1995). Endocrine and other biological rhythms. Endocrinology. L. J. DeGroot. Philadelphia, Saunders: 2487-2548.
- Van Cauter, E., Leproult, R. and Kupfer, D. J. (1996). "Effects of Gender and age on the levels and circadian rhythmicity of plasma cortisol." Journal Clinical Endocrinology Metabolism 81: 2468-2473.
- Van Eck, M. (1996). "The effects of perceived stress, traits, mood states, and stressful daily events on salivary cortisol." Psychosomatic Medicine 58: 447-458.
- Van Gelder, B. M., Tijhuis, M. A. R., Kalmijn, S., Giampaoli, S., Nissinen, A. and Kromhout, D. (2004). "Physical activity in relation to cognitive decline in elderly men. The FINE study." Neurology 63: 2316-2321.
- Van Niekerk, J. K., Huppert, F. and Herbert, J. (2001). "Salivary cortisol and DHEA: association with measures of cognition and well being in normal older men, and effects of three months of DHEA supplementation." Psychoneuroendocrinology 26: 591-612.
- Van Stegeren, A. H., Gockoop, R., Everaerd, W., Scheltens, P., Barkhof, F., Kuijjer, J. P. A. and Rombouts, A. R. B. (2005). "Noradrenaline mediates amygdala activation in men and women during encoding of emotional material." NeuroImage 24: 898-909.
- Van Strien, T., Frijters, J. E., Bergers, G. P. and Defares, P. B. (1986). "The Dutch Eating Behaviour Questionnaire (DEBQ) for assessment of restrained, emotional, and external eating behaviour." International Journal of Eating Disorders 5(2): 295-315.
- Vanhanen, M., Koivisto, K., Kuusisto, J., Mykkanen, L., Helkala, E. L. and Hanninen, T. (1998). "Cognitive function in an elderly population with persistent impaired glucose tolerance." Diabetes Care 21: 398-402.
- Vgontzas, A. N., Mastorakos, G., Bixler, E. O., Kales, A., Gold, P. W. and Chrousos, G. P. (1999). "Sleep deprivation effects on the activity of the hypothalamic-pituitary-adrenal and growth axes: potential clinical implications." Clinical Endocrinology 51: 205-215.
- Viau, V. and Meaney, M. J. (1991). "Variations in the hypothalamic-pituitary-adrenal response to stress during the estrous cycle in the rat." Endocrinology 129: 2503-2511.
- Vincennati, V. and Pasquali, R. (2000). "Abnormalities of the hypothalamic pituitary-adrenal axis in non depressed women with abdominal obesity and relations with insulin resistance: evidence for a central and a peripheral alteration." Journal Clinical Endocrinology Metabolism 85: 4093-4098.



- Vining, R. F., McGinley, R. A. and Symons, R. G. (1983). "Hormones in saliva: mode of entry and consequent implications for clinical interpretation." Clinical Chemistry **29**: 1752-1756.
- Vining, R. F. and McGinley, R. A. (1987). "The measurement of hormones in Saliva: Possibilities and Pitfalls." Journal Steroid Biochemistry **27**(1-3): 81-94.
- Virgin, C. E., Taryn, P. T. H. and Packan, D. R. (1991). "Glucocorticoids inhibit glucose transport and glutamate uptake in the hippocampal astrocytes: implications for glucocorticoid neurotoxicity." Journal Neurochemistry **57**: 1422-1428.
- Visscher, T. L., Seidell, J. C., Molarius, A., van der Kuip, D., Hofman, A. and Witteman, J. C. (2001). "A comparison of body mass index, waist-hip ratio and waist circumference as predictors of all-cause mortality among the elderly: the Rotterdam study." International Journal of Obesity Related Metabolic Disorders **11**: 1730-1735.
- Visser, J., Boxel-Dezairre, A., Methorst, D., Brunt, T., deKloet, E. R. and Nagelkerken, L. (1998). "Differential regulation of interleukin-10 and interleukin-12 by glucocorticoids in vitro." Blood **91**: 4255-64.
- Vogel, W. H. (1985). "Coping, stress, stressors and health consequences." Neuropsychobiology **13**: 129-135.
- Voyer, D., Voyer, S. and Bryden, M. P. (1995). "Magnitude of sex differences in spatial abilities: a meta-analysis and consideration of critical variables." Psychological Bulletin **117**: 250-270.
- Waldstein, S. R. and Katzel, L. (2005). "Stress-Induced Blood Pressure Reactivity and Cognitive Function." Neurology **64**(10): 1746-9.
- Waldstein, S. R. and Katzel, L. (2006). "Interactive relations of central versus total obesity and blood pressure to cognitive function." International Journal of Obesity **30**: 201-207.
- Walker, B. R., Sonderberg, S., Lindhal, B. and Olsson, T. (2000). "Independent effects of obesity and cortisol in predicting cardiovascular risk factors in men and women." Journal International Medicine **247**: 198-204.
- Wallerius, S., Rosmond, R., Ljung, T., Holm, G. and Bjorntorp, P. (2004). "Rise in morning saliva cortisol is associated with abdominal obesity in men: a preliminary report." Journal Endocrinology Investigation **26**: 616-619.
- Ward, A. M., Fall, C. H., Stein, M., Kumaran, K., Veena, S. R., Wood, P. J., Syddall, H. E. and Phillips, D. I. W. (2003). "Cortisol and the metabolic syndrome in South Asians." Clinical Endocrinology Metabolism **58**: 500-505.
- Ward, A. M., Syddall, H. E., Wood, P. J., Dennison, E. M. and Phillips, D. I. W. (2004). "Central hypothalamic-pituitary-adrenal activity and the metabolic syndrome: Studies using the corticotrophin-releasing hormone test." Metabolism - Clinical and experimental **55**(6): 720-726.

- Wardle, J. and Gibson, E. L. (2002). Impact of stress on diet: processes and implications. Stress and the heart psychosocial pathways to coronary heart disease. S. M. Stansfield, M. London, BMJ Books: 124-149.
- Waye, K. P., Agge, A., Clow, A. and Hucklebridge, F. (2004). "Cortisol response and subjective sleep disturbance after low-frequency noise exposure." Journal of Sound and Vibration 277(3): 453-457.
- Weber-Hamann, B., Hentschel, F., Kniest, A., Deuschle, M., Colla, M., Lederbogen, F. and Heuser, I. (2002). "Hypercortisolemic depression is associated with increased intra-abdominal fat." Psychosomatic Medicine 64: 274-277.
- Weber-Hamann, B., Werner, M., Hentschel, F., Bindeballe, N., Lederbogen, F., Deuschle, M. and Heuser, I. (2006). "Metabolic changes in elderly patients with major depression: Evidence for increased accumulation of visceral fat at follow-up." Psychoneuroendocrinology 31: 347-354.
- Weerth, C. and Buitelaar, J. K. (2005). "Cortisol awakening response in pregnant women." Psychoneuroendocrinology 30: 902-907.
- Weigratz, I., Kutschera, E., Lee, J.H., Moore, C., Mellinger, U., Winkler, U. H. and Kuhl, H. (2003). "Effect of four different oral contraceptives on various sex hormones and serum binding globulins." Contraception 67: 25-32.
- Weitzman, E. D., Fukushima, D., Nogeire, C., Roffwarg, C., Gallagher, T. F. and Hellman, L. (1971). "Twenty-four hour pattern of the episodic secretion of cortisol in normal subjects." Clinical Endocrinology Metabolism 33: 14-22.
- Weitzman, E. D., Zimmermann, J. C., Czeisler, C. A. and Ronda, J. (1983). "Cortisol secretion is inhibited during sleep in normal man." Clinical Endocrinology Metabolism 56: 352-360.
- Welborn, T. A., Dhaliwal, S. S. and Bennett, S. A. (2003). "Waist-hip ratio is the dominant risk factor predicting cardiovascular death in Australia." Weighty Issues 179: 580-585.
- West, M. (1993). "Regionally specific loss of neurons in the aging human hippocampus." Physiology of Aging 14: 287-293.
- Westerbaka, J., Yki-Jarvinen, H., Vehkavaara, S., Hakkinen, A., Andrew, R., Wake, J., Seckl, J. R. and Walker, B. (2003). "Body fat distribution and cortisol metabolism in healthy men: Enhanced 5B-Reductase and lower cortisol/cortisone metabolite ratios in men with fatty liver." Journal Clinical Endocrinology Metabolism 88(10): 4924-4931.
- Wetzel, D. M., Bohn, M. C., Kazee, A. M. and Hamill, R. W. (1995). "Glucocorticoid receptor mRNA in Alzheimer's diseased hippocampus." Brain Research 679: 72-81.
- Whaley, M. H., Kampert, J. B., Kohl III, H. W. and Blair, S. N. (1999). "Physical fitness and clustering of risk factors associated with the metabolic syndrome." Medicine and Science in Sports and Exercise 31: 287-293.

- WHO (1999). Report of a WHO consultation: definition of metabolic syndrome in definition, diagnosis, and classification of diabetes mellitus and its complications. I. Diagnosis and classification of diabetes mellitus., Geneva, Department of Noncommunicable Disease Surveillance.
- WHO (2000). The Asia-pacific perspective: redefining obesity and its treatment. World Health Organisation, Geneva.
- WHO (2002). World Health Report: Reducing Risks, Promoting Life. Geneva.
- Whorwood, C. B., Mason, J. W., Ricketts, M. L., Howie, A. J. and Stewart, P. M. (1995). "Detection of human 11 $\beta$  - Hydroxysteroid Dehydrogenase isoforms using the reverse-transcriptase-polymerase chain reaction and localization of the type 2 isoform to renal collecting ducts." Mol Cell Endocrinol **110**(R7-12).
- Williams, D. P., Boyden, T. W. and Pamerter, R. W. (1993). "Relationship of body fat percentage and fat distribution with DHEAS in pre-menopausal females." Journal Clinical Endocrinology Metabolism **77**: 80-85.
- Williams, E., Magid, K. and Steptoe, A. (2005). "The impact of time of waking and concurrent subjective stress on the cortisol response to awakening." Psychoneuroendocrinology **30**: 139-148.
- Williamson, M., Bingham, B. and Viau, V. (2005). "Central organisation of androgen-sensitive pathways to the hypothalamic-pituitary-adrenal axis: Implications for the individual differences in responses to homeostatic threat and predisposition to disease." Progress in Neuro-Psychopharmacology & Biological psychiatry. **29**: 1239-1248.
- Wolf, O. T., Koster, B., Kirschbaum, C., Pietrowsky, R., Kern, W., Hellhammer, D., Born, J. and Fehm, H. L. (1997). "A single administration of Dehydroepiandrosterone does not enhance memory performance in young healthy adults, but immediately reduces cortisol levels." Biological Psychiatry **42**: 845-848.
- Wolf, O. T., Kudielka, B. M., Hellhammer, D., Hellhammer, J. and Kirschbaum, C. (1998). "Opposing effects of DHEA replacement in elderly subjects on declarative memory and attention after exposure to a laboratory stressor." Psychoneuroendocrinology **23**(6): 617-629.
- Wolf, O. T., Schommer, N. C., Hellhammer, D. H., McEwen, B. S. and Kirschbaum, C. (2001). "The relationship between stress induced cortisol levels and memory differs between men and women." Psychoneuroendocrinology **26**: 711-720.
- Wolf, O. T., Convit, A., Thorn, E. and de Leon, M. J. (2002). "Salivary cortisol day profiles in elderly with mild cognitive impairment." Psychoneuroendocrinology **27**(7): 777-789.
- Wolf, O. T., Dziobek, I., McHugh, P., Sweat, V., de Leon, M. J., Javier, E. and Convit, A. (2005). "Subjective memory complaints in aging are associated with elevated cortisol levels." Neurobiology of Aging **26**: 1357-1363.

- Wolf, O. T. and Kuhlmann, S. (2006). Emotional arousal induced by the learning material or the testing conditions modifies the effect of cortisol on memory in humans. 37th Annual International Society for Psychoneuroendocrinology (ISPNE) Conference: Hormones and Brain from Cloning to Clinic, Leiden, The Netherlands.
- Wolkowitz, O. M., Reus, V. I., Weingartner, H., Thompson, K., Breier, A., Doran, A., Rubinow, D. and Pickar, D. (1990). "Cognitive effects of corticosteroids." American Journal Psychiatry **147**: 1297-1303.
- Wolkowitz, O. M., Weingartner, H., Rubinow, D., Jimerson, D., Kling, M., Berrentini, W., Thompson, K., Breier, A., Doran, A., Reus, V. I. and Pickar, D. (1993). "Steroid modulation of human memory: biochemical correlates." Biological Psychiatry **33**: 744-746.
- Wood, G. E. and Shors, T. J. (1998). "Stress facilitates classical conditioning in males, but impairs classical conditioning in females through activational effects of ovarian hormones." National Academic Science. USA **95**: 4066-4071.
- Wright, C. E. and Steptoe, A. (2005). "Subjective socioeconomic position, gender and cortisol responses to waking in an elderly population." Psychoneuroendocrinology **30**: 582-590.
- Wu, C. C., Mungas, D. and Petkov, C. L. (2002). "Brain structure and cognition in a community sample of elderly Latinos." Neurology **59**: 383-391.
- Wüst, S., Wolf, J., Hellhammer, D. H., Federenko, I., Schommer, N. and Kirschbaum, C. (2000b). "The cortisol response to awakening-normal values and confounds." Noise and Health **7**: 77-85.
- Wüst, S., Federenko, I., Hellhammer, D. H. and Kirschbaum, C. (2000). "Genetic factors, perceived chronic stress, and the free cortisol response to awakening." Psychoneuroendocrinology **25**: 707-720.
- Wüst, S., van Rossum, E. F. C., Federenko, I., Koper, J. W., Kumsta, R. and Hellhammer, D. (2004). "Common polymorphisms in the Glucocorticoid Receptor gene are associated with adrenocortical responses to psychological stress." Journal of Clinical Endocrinology and Metabolism **89**(2): 565-573.
- Wüst, S., Federenko, I., van Rossum, E. F. C., Koper, J. W. and Hellhammer, D. H. (2005). "Habituation of cortisol responses to repeated psychosocial stress-further characterization and impact of genetic factors." Psychoneuroendocrinology **30**: 199-211.
- Yamaguchi, Y., Tanaka, S., Yamakawa, T., Kimura, M., Ukawa, K., Yamada, Y., Ishihara, M. and Sekihara, H. (1998). "Reduced serum dehydroepiandrosterone levels in diabetic patients with hyperinsulinemia." Clinical Endocrinology Metabolism **49**: 377-383.
- Yau, J. L. W. and Seckl, J. R. (1995). "Corticosteroids and the brain." Current opinion in Endocrinology and diabetes **2**: 239-247.

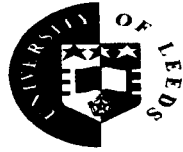
- Yoshizumi, T., Nakamura, T., Yamane, M., Islam, A. H., Menju, M. and Yamasaki, K. (1999). "Abdominal fat: standardized technique for measurement at CT." Radiology **211**: 283-286.
- Young, A. H., Sahakian, B. J., Robbins, T. W. and Cowen, P. J. (1999). "The effects of chronic administration of hydrocortisone on cognitive function in normal male volunteers." Psychopharmacology **145**: 260-266.
- Zeineh, M. M., Engel, S. A., Thompson, P. M. and Bookheimer, S. Y. (2003). "Dynamics of the hippocampus during encoding and retrieval of face-name pairs." Science **299**: 577-580.
- Zeise, M. L., Teschemacher, A., Arriagada, J. and Zieglansberger, W. (1992). "Corticosterone reduces synaptic inhibition in rat hippocampal and neocortical neurons in vitro." J. Neuroendocrinology **4**: 107-118.
- Zisapel, N. and Laudon, M. (2003). "Subjective assessment of the effects of CNS-active drugs on sleep by the Leeds sleep evaluation questionnaire: a review." Human Psychopharmacology **18**: 1-20.

**Appendices**

---

---

## Appendix 1: Volunteer Screening Booklet



## PARTICIPANT SCREENING PACK

Thank you for your decision to participate in this study. The following information pack is designed to collect some data about yourself, your health and well being, in order to assess your suitability for this study and to determine what benefits participating may hold for you.

You are under *no obligation* to answer any question that you may not wish to answer for any reason, but are reminded that your responses will help us to help you and will aid the understanding of you as a person. Your confidentiality will be respected at ALL times.

Thank you

## Section One: About You

1. Name: .....

Date of Birth:   /   /

Age:

2. Gender: MALE \ FEMALE (Please circle)

3. Are you currently: (please tick)

Single	<input type="checkbox"/>
Married	<input type="checkbox"/>
Living with partner	<input type="checkbox"/>
Divorced and single	<input type="checkbox"/>
Divorced and remarried	<input type="checkbox"/>
Widowed and remarried	<input type="checkbox"/>
Widowed and single	<input type="checkbox"/>

If you are in a relationship, how long?  years

Do you have any children? (Please tick)

No

Yes  (...number of children)

**Section Two: Your Employment**

1. Are you? (Please tick)

- Employed – full time
- Employed –part time
- Unemployed
- Student
- Housewife
- Retired

2. What is / was your most recent employment?

---



---



---

3. Did / does this position require that you work any abnormal shifts e.g. night shifts?

YES / NO (Please circle)

If 'YES' please specify:

---



---



---

**Section 3: Your Diet**

1. Are you currently dieting to lose weight?

YES / NO

2. Do you avoid any specific foods?

YES / NO

If 'YES', which foods do you avoid?

---



---

- |  |                  |                          |
|--|------------------|--------------------------|
| Why do you avoid this / these food(s)? | Slimming         | <input type="checkbox"/> |
|  | Allergic         | <input type="checkbox"/> |
|  | Health           | <input type="checkbox"/> |
|  | Religion         | <input type="checkbox"/> |
|  | Dislike the food | <input type="checkbox"/> |



Vegetarian

3. How would you rate your diet? (Please indicate with a vertical line)

Not at all  
Healthy

\_\_\_\_\_

Extremely  
Healthy

**Section 4: Your Health**

1. How would you rate your general health? (Please indicate with a vertical line)

Not at all  
healthy

\_\_\_\_\_

Extremely  
Healthy

2. Do you smoke?

No, have never smoked

No, have given up

(How Long? .....years)

Yes, occasionally

Yes, regularly

3. Are you currently on any form of medication? If so please detail below:

Medication

What is it for?

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**If you are MALE, please skip to question 5**

4. Please tick the statement which best applies to you;

I have not had a period for 2 years	<input type="checkbox"/>
I have not had a period for 1 year	<input type="checkbox"/>
I still have some periods but they are irregular and erratic	<input type="checkbox"/>
I still have regular periods	<input type="checkbox"/>

Have you experienced hot flushes? (Please tick)

Yes, frequently	<input type="checkbox"/>
Sometimes	<input type="checkbox"/>
Rarely	<input type="checkbox"/>
Never	<input type="checkbox"/>

If you still have regular periods;

What method of contraception do you use? *(Please tick)*

- None
- Condom / Femidom
- Diaphragm / Cap
- Spermicidal cream (cream, foam, sponge)
- Rhythm method (taking temperature, counting)
- Withdrawal (coitus interruptus)
- Combined pill
- Progesterone only pill (or mini pill)
- Implants (e.g. Norplant)   
Which? \_\_\_\_\_
- Coil / IUD:   
Which? \_\_\_\_\_
- Contraceptive injections   
Which? \_\_\_\_\_
- Hysterectomy
- Sterilised
- Partner is sterilised
- Past menopause (\_\_\_\_\_ years ago)

If you use another form of contraception, please specify below:

\_\_\_\_\_

Are you currently taking/have taken any form of Hormone Replacement Therapy?

- No
- Yes, ..... Years ago
- Yes, take it at present   
If 'YES', what is / was it called?

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

What was the date of your last period?      \_\_ / \_\_ / \_\_

How many days do you usually bleed for? e.g. 5      \_\_ Days

How long is your average cycle? i.e. how many days from the start of one period to the start of the next e.g. 29 days  
 \_\_ Days

5. Have you ever suffered from any of the following in the past or at present (currently)? (Please tick if applicable)

- Asthma
- Diabetes
- High blood pressure
- Low blood pressure
- Any cardiac (heart) problems
- Hormonal problems
- Digestive disorders/problems
- Liver problems
- Neurological problems (nervous system disorders)
- Mental problems E.g. depression, anxiety etc
- Any type of eating disorder

Past	Currently

**Section 5: Exercise**

1. What forms of exercise do you do? (Please tick all that apply)

- |          |                          |                              |                          |
|----------|--------------------------|------------------------------|--------------------------|
| Running  | <input type="checkbox"/> | Gym Use                      | <input type="checkbox"/> |
| Walking  | <input type="checkbox"/> | Other (please specify below) | <input type="checkbox"/> |
| Cycling  | <input type="checkbox"/> | .....                        |                          |
| Swimming | <input type="checkbox"/> | .....                        |                          |
| Aerobics | <input type="checkbox"/> | .....                        |                          |

2. Do you play sport? ..... YES/NO. If yes.....

- Which sport do you play most frequently? \_\_\_\_\_

- How many hours a week?..... (Circle).... <1    1-2    2-3    3-4    >4

- How many months a year? ..... (Circle).... <1    1-3    4    7-9    >9

- If you play a second sport, what is it? \_\_\_\_\_

- How many hours a week? ..... (Circle).... <1    1-2    2-3    3-4    >4

- How many months a year? ..... (Circle).... <1    1-3    4-6    7-9    >9

3. In comparison with others of my own age I think my physical activity during leisure time is: (please circle)

Much less

less

the same

more

much more

THANK YOU FOR YOUR TIME

If you have any comments or queries about this questionnaire or your participation in this study then please do not hesitate to contact me:

Nicola Lasikiewicz  
Tel: 0113 343 6697  
Email: [pscnjl@leeds.ac.uk](mailto:pscnjl@leeds.ac.uk)

## Appendix 2: Telephone Screening Questionnaire

Telephone Interview.

Date:

Time:

Ppt name:

Age/DOB:

M / F (circle)

Contact Details:

Tel num:

Email:

Address (optional):

Where did you hear about this study?

A few preliminary questions:

Do you smoke? Y / N

Are you currently on any form of medication e.g. steroidal, anti-inflammatory, hormonal medication etc? Y / N

If yes, please indicate what this medication is for (optional):

**IF FEMALE** Are you currently taking any form of contraception, implants or HRT treatment at the moment? Y / N

What is your occupation?

Does this job require that you work any night or abnormal shifts? Y / N

Have you given blood in the past few months? Y / N

Thank the caller and inform the candidate that the researcher will be in touch shortly with further details.

Office Use Only

Selected for participation: Y / N

Participant informed? Y / N

Date:

Screening pack provided: Y / N

Date:

Participant assigned code: EXINL-\_\_\_\_\_

Authorisation: \_\_\_\_\_

### Appendix 3: Saliva Sampling Guide 1

Date: \_\_/\_\_/\_\_

Participant Identification: EX2NL - \_\_\_\_

Sample Day:      Monitoring Day One    Test day Monitoring Day Two    (please circle)

#### SAMPLE GUIDE

PLEASE ENTER YOUR WAKING TIME FOR THIS MORNING IN THE SPACE BELOW:

Time: \_\_: \_\_ am/pm (delete as appropriate)

PLEASE COMPLETE THE TIME SLOTS BELOW BASED ON YOUR WAKING TIME.  
IT IS IMPORTANT THAT YOU TAKE EACH SAMPLE ON TIME AND MAKE SURE THAT THE  
COTTON WOOL IS SATURATED WITH SALIVA BEFORE RETURNING TO THE TUBE.

TIME	SAMPLE TO BE TAKEN	COMMENTS
What time did you wake up? __: __	D1 - FIRST C1	Remain in bed and keep movements to a minimum. Use Drool pot first – generate as much saliva as possible and drool into pot Use the salivette IMMEDIATELY following this. Nil by Mouth except water if necessary and refrain from brushing teeth.
15 minutes post waking: __: __	C2	Remain in bed and keep movements to a minimum. Nil by mouth except water if necessary. Refrain from brushing teeth.
30 minutes post waking: __: __	C3	Remain in bed and keep movements to a minimum. Nil by mouth except water if necessary. Refrain from brushing teeth.
45 minutes post waking: __: __	C4	Nil by mouth except water if necessary and refrain from brushing teeth until after sample has been taken. Resume normal activities after this sample.
3 hours post waking: __: __	C5	Nil by mouth 30minutes before the sample is due to be taken.
6 hours post waking: __: __	C6	Nil by mouth 30minutes before the sample is due to be taken.
9 hours post waking: __: __	C7	Nil by mouth 30minutes before the sample is due to be taken.
12 hours post waking: __: __	C8	Nil by mouth 30minutes before the sample is due to be taken.

**Appendix 4: Saliva Sample Guide 2**

Date: \_\_\_/\_\_\_/\_\_\_

Participant Identification: EX2NL - \_\_\_

Sample Day:      Monitoring Day One    Test day Monitoring Day Two    (please circle)

SAMPLE GUIDE

PLEASE ENTER YOUR WAKING TIME FOR THIS MORNING IN THE SPACE BELOW:

Time: \_\_\_:\_\_\_ am/pm (delete as appropriate)

PLEASE COMPLETE THE TIME SLOTS BELOW BASED ON YOUR WAKING TIME.  
IT IS IMPORTANT THAT YOU TAKE EACH SAMPLE ON TIME AND MAKE SURE THAT THE  
COTTON WOOL IS SATURATED WITH SALIVA BEFORE RETURNING TO THE TUBE.

TIME	SAMPLE TO BE TAKEN	COMMENTS
What time did you wake up?  :	D1 - FIRST C1	Remain in bed and keep movements to a minimum. Use Drool pot first – generate as much saliva as possible and drool into pot Use the salivette IMMEDIATELY following this. Nil by Mouth except water if necessary and refrain from brushing teeth.
15 minutes post waking:  :	C2	Remain in bed and keep movements to a minimum. Nil by mouth except water if necessary. Refrain from brushing teeth.
30 minutes post waking:  :	C3	Remain in bed and keep movements to a minimum. Nil by mouth except water if necessary. Refrain from brushing teeth.
45 minutes post waking:  :	C4	Nil by mouth except water if necessary and refrain from brushing teeth until after sample has been taken. Resume normal activities after this sample.

**Appendix 5: Salivette Instruction Sheet**Instructions for Saliva Sampling using Salivettes.

1. Collect saliva at the times specified on your Daily Sampling Schedule. Saliva should be collected no earlier than 30 minutes after eating or drinking.
2. Hold the salivette at the rim of the suspended insert and remove the stopper. Removal of the stopper is easier if you slightly push it to the side when opening the suspended insert.
3. Now remove the cotton wool swab from the salivette.
4. Collect the saliva by chewing the cotton wool. Keep the cotton in your mouth until you feel that you can no longer prevent yourself from swallowing the saliva produced. This normally takes about 30-45 seconds.
5. Now return the saturated cotton wool swab to the suspended insert and close the salivette firmly with the stopper.
6. Until you have collected all samples, keep the salivettes refrigerated until they can be returned to the researcher.

If you have any problems or queries, please contact Nicola on 0113 343 6697 or [pscnjl@leeds.ac.uk](mailto:pscnjl@leeds.ac.uk).



**Appendix 6: Physiological Data Record Sheet**

Ppt ID: EX1NL \_\_\_

Date: \_\_/\_\_/\_\_

Participant Physiological Data

Blood sample Obtained? YES / NO

Difficult venepuncture? YES / NO

Comments: \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

Height (cm)						
Weight (kg)						
BMI						
Waist (cm)						
Hip (cm)						
Waist Hip Ratio						
Pulse						
Blood Pressure	1.	2.	3.	4.	5.	Average:
Body Composition performed? YES / NO (attached copy to this document)						
Body Comp	% Fat:		Fat Mass:			

**Thank the Participant**

**Appendix 7: Colour Blindness Test**

VOLUNTEER ID: EX2NL: \_\_\_

DATE: \_\_\_/\_\_\_/\_\_\_

COLOUR BLINDNESS

Please indicate the colour ink each word is printed in using the spaces below.

Thank you.

COLOUR	COLOUR	COLOUR	COLOUR
--------	--------	--------	--------

1. Please indicate using a vertical line on the scale below how easy/difficult you felt it was to complete each of the following aspects:

(a) preparation

Very well ----- not very well

-----

2. Please indicate using a vertical line on the scale below how easy/difficult you felt it was to complete each of the following aspects:

(a) preparation

Easy ----- Difficult

-----

(b) in the interview itself

Easy ----- Difficult

-----

(c) in the mental arithmetic task

Easy ----- Difficult

-----

3. (a) How easy/difficult did you find the cognitive tests?

Very well ----- not very well

-----

(b) How well do you think you have done in the cognitive tests?

Very well ----- not very well

-----

4. Please indicate using a vertical line on the scale below how stressful you felt each of the following aspects was for you:

(a) preparation

Very stressful ----- not at all stressful

-----

(b) the interview (including the mental arithmetic task)

Very stressful ----- not at all stressful

-----

(c) the cognitive tests

Very stressful ----- not at all stressful

-----

## Appendix 8: Visual Analogue Scales (Subjective Evaluation/Debrief Questionnaire)

### Version for Stress Condition

PARTICIPANT ID: EX2NL- \_\_\_

DATE: \_\_\_/\_\_\_/\_\_\_

#### DEBRIEF QUESTIONNAIRE

Please answer the following questions about the experiment you have just taken part in. Thank you.

1. Please indicate using a vertical line on the scale below how well you feel you did in the interview task for each of the following aspects:

(a) preparation

Very well \_\_\_\_\_ not very well

(b) in the interview itself

Very well \_\_\_\_\_ not very well

(c) in the mental arithmetic task

Very well \_\_\_\_\_ not very well

2. Please indicate using a vertical line on the scale below how easy/difficult you felt it was to complete each of the following aspects:

(a) preparation

Easy \_\_\_\_\_ Difficult

(b) in the interview itself

Easy \_\_\_\_\_ Difficult

(c) in the mental arithmetic task

Easy \_\_\_\_\_ Difficult

3. (a) How easy/difficult did you find the cognitive tests?

Very well \_\_\_\_\_ not very well

(b) How well do you think you have done in the cognitive tests?

Very well \_\_\_\_\_ not very well

4. Please indicate using a vertical line on the scale below how stressful you felt each of the following aspects was for you:

(a) preparation

Very stressful \_\_\_\_\_ not at all stressful

(b) the interview (including the mental arithmetic task)

Very stressful \_\_\_\_\_ not at all stressful

(c) the cognitive tests

Very stressful \_\_\_\_\_ not at all stressful

**Version for No Stress Condition:**

PARTICIPANT ID: EX2NL- \_\_\_\_

DATE: \_\_/\_\_/\_\_

**DEBRIEF QUESTIONNAIRE**

Please answer the following questions about the experiment you have just taken part in. Thank you.

1. Please indicate using a vertical line on the scale below how well you feel you did in the preparing your application letter?

Very well \_\_\_\_\_ not very well

2. Please indicate using a vertical line on the scale below how easy/difficult you felt it was to prepare your application letter?

Easy \_\_\_\_\_ Difficult

3. (a) How easy/difficult did you find the cognitive tests?

Easy \_\_\_\_\_ Difficult

(b) How well do you think you have done in the cognitive tests?

Very well \_\_\_\_\_ not very well

4. Please indicate using a vertical line on the scale below how stressful you felt each of the following aspects was for you:

(a) preparing the application letter:

Very stressful \_\_\_\_\_ not at all stressful

(b) talking about the application letter:

Very stressful \_\_\_\_\_ not at all stressful

(c) the cognitive tests

Very stressful \_\_\_\_\_ not at all stressful

**Appendix 9: Post Stress Study Debrief Sheet**  
**Version for Stress Condition**

PARTICIPANT INFORMATION SHEET – DEBRIEF

Thank you for taking part in this research. Now that the experiment is complete, I would like to provide with some information the study and the types of task you have completed and what they were for.

This study was an examination of the impact of stress induced cortisol activity on cognition, i.e. your memory, attention and concentration. It also aimed to examine differences in stress responsivity (how you responded to the stressor in terms of cortisol, blood pressure and mood) in terms of body shape (apples vs. pears).

You were placed in the 'stress' condition. The interview scenario of which you took part is a version of what is known as the Trier Social Stress Test (TSST). This is a widely used standardised laboratory stress induction tool pioneered by researchers at the University of Trier in Germany. This served as a stressor in the form of a public speaking task in an attempt to increase your cortisol levels and effectively to induce a stress 'response'. Once a response was initiated it was then possible to examine whether an increase in stress would affect your ability to remember information, to pay attention to certain stimuli, maintain concentration, plan moves in a sequence and solve problems in basic computer tasks. The tests that you completed form part of the widely used and reliable Cambridge Automated Neuropsychological Test Battery or CANTAB. This is comprises a number of tests administered to assess various aspects of memory and mental performance.

By conducting this research it is hopeful that an insight will be gained into how we cope mentally during stressful situations, are we able to remember information and pay attention when under stress. Of particular interest is the action of the stress hormone cortisol, often found to influence cognitive performance. Cortisol is also linked to body shape, and often we see that levels of circulating cortisol are altered in those with a high waist hip ratio (apple shaped). By exploring the impact body shape has on our responses to stress we can hopefully see if any one individual is more vulnerable to the effects of stress both on health and on cognition. With this knowledge it may be possible to aid further research into combating the more negative aspects of stress and improving quality of life. Once all data has been collected and the results processed a special feedback evening will be arranged for all who took part. Details will be available nearer the time. Should you have any questions or comments then please contact me using the details below.

THANK YOU

Email: [pscnjl@leeds.ac.uk](mailto:pscnjl@leeds.ac.uk)

Telephone: 0113 343 6697

**Version for No Stress Condition:**PARTICIPANT INFORMATION SHEET – DEBRIEF

Thank you for taking part in this research. Now that the experiment is complete, I would like to provide with some information the study and the types of task you have completed and what they were for.

This study was an examination of the impact of stress induced cortisol activity on cognition, i.e. your memory, attention and concentration. It also aimed to examine differences in stress responsivity (how you responded to the stressor in terms of cortisol, blood pressure and mood) in terms of body shape (apples vs. pears).

You were placed in the 'no stress' condition. Preparing an application letter served as a control condition to the Trier Social Stress Test (TSST) a procedure used to initiate a stress response so that the impact of elevated stress levels on cognitive performance can be examined. In the no stress condition your ability to complete various cognitive tasks such as remembering information, paying attention to certain stimuli, maintaining concentration, planning moves in a sequence and solving problems in basic computer tasks was examined under 'normal' or 'control' conditions. The tests that you completed form part of the widely used and reliable Cambridge Automated Neuropsychological Test Battery or CANTAB. This is comprises a number of tests administered to assess various aspects of memory and mental performance.

By conducting this research it is hopeful that an insight will be gained into how we cope mentally during stressful situations, are we able to remember information and pay attention when under stress. Of particular interest is the action of the stress hormone cortisol, often found to interact with cognitive performance. Cortisol is also linked to body shape, and often we see that levels of circulating cortisol are altered in those with a high waist hip ratio (apple shaped). By exploring the impact body shape has on our responses to stress we can hopefully see if any one individual is more vulnerable to the effects of stress both on health and on cognition. With this knowledge it may be possible to aid further research into combating the more negative aspects of stress and improving quality of life. Once all data has been collected and the results processed a special feedback evening will be arranged for all who took part. Details will be available nearer the time. Should you have any questions or comments then please contact me using the details below.

THANK YOU

Email: [pscnjl@leeds.ac.uk](mailto:pscnjl@leeds.ac.uk)

Telephone: 0113 343 6697

### Appendix 10: Perceived Stress Scale (PSS)

#### Perceived Stress Scale

The questions in this scale ask you about your feelings and thoughts during the **last month**. In each case, you will be asked to indicate by circling *how often* you felt or thought a certain way.

**0=Never    1=Almost Never    2=Sometimes    3=Fairly often    4=Very often**

1. In the last month, how often have you been upset about something that has happened unexpectedly?	0	1	2	3	4
2. In the last month, how often have you felt that you were unable to control the important things in your life?	0	1	2	3	4
3. In the last month, how often have you felt nervous and 'stressed'?	0	1	2	3	4
4. In the last month, how often have you felt confident about your ability to handle your personal problems?	0	1	2	3	4
5. In the last month, how often have you felt that things were going your way?	0	1	2	3	4
6. In the last month, how often have you found that you could not cope with all the things that you had to do?	0	1	2	3	4
7. In the last month, how often have you been able to control the irritations in your life?	0	1	2	3	4
8. In the last month, how often have you felt that you were on top of things?	0	1	2	3	4
9. In the last month, how often have you been angered because of things that were outside your control?	0	1	2	3	4
10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?	0	1	2	3	4

### Appendix 11: Leeds Sleep Evaluation Questionnaire (LSEQ)

Ppt ID: EX2NL - \_\_\_\_\_

DAY: 1 2 3 (circle)

Time: \_\_ : \_\_ AM/PM

#### Sleep

Each of the following questions are about a **TYPICAL night's sleep**. Answer the questions by placing a vertical mark through the line in the place that best indicates your answer.

1. In a typical night how EASY is it to get to sleep?

very easy \_\_\_\_\_ very difficult

2. How QUICKLY do you get to sleep?

very quickly \_\_\_\_\_ very slowly

3. What is the QUALITY of your sleep in a typical night?

very restful \_\_\_\_\_ not at all restful

no periods of wakefulness \_\_\_\_\_ many periods of wakefulness

4. What is your pattern of AWAKENING like in a typical morning?

very easy \_\_\_\_\_ very difficult

takes a short time \_\_\_\_\_ takes a long time

5. How do you usually FEEL ON AWAKENING?

alert \_\_\_\_\_ tired

6. How do you usually feel 1 HOUR AFTER awakening?

alert \_\_\_\_\_ tired



### Sleep – Section 2

Each of the following questions are about **LAST night's sleep**. Answer the questions by placing a vertical mark through the line in the place that best indicates your answer.

1. How EASY was it to get to sleep last night?

very easy \_\_\_\_\_ very difficult

2. How QUICKLY did you get to sleep last night?

very quickly \_\_\_\_\_ very slowly

3. What was the QUALITY of your sleep last night?

very restful \_\_\_\_\_ not at all restful

no periods of wakefulness \_\_\_\_\_ many periods of wakefulness

4. What was your pattern of AWAKENING like this morning?

very easy \_\_\_\_\_ very difficult

took a short time \_\_\_\_\_ took a long time

5. How did you FEEL ON AWAKENING this morning?

alert \_\_\_\_\_ tired

6. How did you feel 1 HOUR AFTER awakening this morning?

alert \_\_\_\_\_ tired

## Appendix 12: Hospital Anxiety and Depression Scale (HADS)

### Hospital Anxiety and Depression Scale (HADS)

Please read each item and place a tick opposite the reply which comes closest to how you have been feeling in the past few weeks. Remember that your immediate reaction to each item may be more accurate than a long thought-out response.

1. I feel tense or 'wound up'

Most of the time  
A lot of the time  
Time to time, occasionally  
Not at all

<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>

2. I feel as if I am slowed down:

Nearly all the time  
Very often  
Sometimes  
Not at all

<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>

3. I still enjoy the things I used to enjoy:

Definitely as much  
Not quite so much  
Only a little  
Hardly at all

<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>

4. I get a sort of frightened feeling like 'butterflies' in the stomach:

Not at all  
Occasionally  
Quite often  
Very often

<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>

5. I get a sort of frightened feeling as if something awful is about to happen:

Very definitely and quite badly  
Yes but not too badly  
A little but it doesn't worry me  
Not at all

<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>

6. I have lost interest in my appearance

Definitely  
I don't take so much care as I should  
I may not take quite as much care  
I take just as much care as ever

<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>

7. I can laugh and see the funny side of things:

As much as I always could  
Not quite so much now  
Definitely not so much now  
Not at all

<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>

8. I feel restless as if I have to be on the move:

Very much indeed  
Quite a lot  
Not very much  
Not at all

<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>

9. Worrying thoughts go through my mind:

A great deal of the time  
A lot of the time  
From time to time but not too often  
Only occasionally

<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>

10. I look forward with enjoyment to things:

As much as I ever did  
Rather less than I used to  
Definitely less than I used to  
Hardly at all

<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>

11. I feel cheerful:

Not at all  
Not often  
Sometimes  
Most of the time

<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>

12. I get sudden feelings of panic:

Very often indeed  
Quite often  
Not very often  
Not at all

<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>

13. I can sit at ease and feel relaxed:

Definitely  
Usually  
Not often  
Not at all

<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>

14. I can enjoy a good book or radio or TV programme:

Often  
Sometimes  
Not often  
Very seldom

<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>

### Appendix 13: Dutch Eating & Behaviour Scale (DEBQ)

#### Dutch Eating and Behaviour Scale (DEBQ)

Please answer the following questions as carefully and honestly as possible. Read each question and simply circle the number which best applies to you.

0=Never      1= Seldom      2=Sometimes      3=Often      4=very often

1. If you have put on weight, do you eat less than you normally do?	0	1	2	3	4
2. Do you have a desire to eat when you are irritated?	0	1	2	3	4
3. If food tastes good to you, do you eat more than you usually do?	0	1	2	3	4
4. Do you try to eat less at meal times than you would like to eat?	0	1	2	3	4
5. Do you have a desire to eat when you have nothing to do?	0	1	2	3	4
6. Do you have a desire to eat when you are 'fed-up'?	0	1	2	3	4
7. If food smells good and looks good, do you eat more than you usually do?	0	1	2	3	4
8. How often do you refuse food and drink offered because you are concerned about your weight?	0	1	2	3	4
9. Do you have a desire to eat when you are feeling lonely?	0	1	2	3	4
10. If you see or smell something delicious do you have a desire to eat it?	0	1	2	3	4
11. Do you watch exactly what you eat?	0	1	2	3	4
12. Do you have a desire to eat when someone disappoints you?	0	1	2	3	4
13. If you have something delicious to eat, do you eat it straight away?	0	1	2	3	4
14. Do you deliberately eat foods that are slimming?	0	1	2	3	4
15. Do you have a desire to eat when you are cross?	0	1	2	3	4
16. Do you have a desire to eat when you are expecting something to happen?	0	1	2	3	4
17. If you walk past the baker, do you have the desire to buy something delicious?	0	1	2	3	4
18. When you have eaten too much do you eat less than usual on the following days?	0	1	2	3	4
19. Do you get a desire to eat when you are anxious, worried or tense?	0	1	2	3	4
20. If you walk past a snack bar or café do you have a desire to buy something delicious?	0	1	2	3	4
21. Do you deliberately eat less in order not to become heavier?	0	1	2	3	4
22. Do you have a desire to eat when things go against you or when things have gone wrong?	0	1	2	3	4
23. If you see others eating, do you also have a desire to eat?	0	1	2	3	4
24. How often do you try not to eat between meals because you are watching your weight?	0	1	2	3	4
25. Do you have a desire to eat when you are frightened?	0	1	2	3	4
26. Can you resist eating delicious foods?	0	1	2	3	4
27. How often in the evening do you try not to eat because you are watching your weight?	0	1	2	3	4
28. Do you have a desire to eat when you are disappointed?	0	1	2	3	4
29. Do you eat more than usual when you see others eating?	0	1	2	3	4
30. Do you think about you weigh before deciding how much to eat?	0	1	2	3	4
31. Do you have a desire to eat when you are upset?	0	1	2	3	4
32. When you see someone preparing a meal, does it make you want to eat something?	0	1	2	3	4
33. Do you have a desire to eat when you are bored or restless?	0	1	2	3	4

**Appendix 14: National Adult Reading Test (List of words)**

<b>CHORD</b>	<b>SUPERFLUOUS</b>
<b>ACHE</b>	<b>SIMILE</b>
<b>DEPOT</b>	<b>BANAL</b>
<b>AISLE</b>	<b>QUADRUPED</b>
<b>BOUQUET</b>	<b>CELLIST</b>
<b>PSALM</b>	<b>FAÇADE</b>
<b>CAPON</b>	<b>ZEALOT</b>
<b>DENY</b>	<b>DRACHM</b>
<b>NAUSEA</b>	<b>AEON</b>
<b>DEBT</b>	<b>PLACEBO</b>
<b>COURTEOUS</b>	<b>ABSTEMIOUS</b>
<b>RAREFY</b>	<b>DÉTENTE</b>
<b>EQUIVOCAL</b>	<b>IDYLL</b>
<b>NAÏVE</b>	<b>PUERPERAL</b>
<b>CATACOMB</b>	<b>AVER</b>
<b>GAOLED</b>	<b>GAUCHE</b>
<b>THYME</b>	<b>TOPIARY</b>
<b>HEIR</b>	<b>LEVIATHAN</b>
<b>RADIX</b>	<b>BEATIFY</b>
<b>ASSIGNATE</b>	<b>PRELATE</b>
<b>HIATUS</b>	<b>SIDEREAL</b>
<b>SUBTLE</b>	<b>DEMENSE</b>
<b>PROCREATE</b>	<b>SYNCOPE</b>
<b>GIST</b>	<b>LABILE</b>
<b>GOUGE</b>	<b>CAMPANILE</b>

### Appendix 15: Daily Hassles Scale with Self-Report Section

Ppt ID: EX1NL: \_\_\_

Date: \_\_/\_\_/\_\_

#### Daily Hassles Questionnaire

Please read each item and circle the appropriate number in terms of how much the item was a hassle to you TODAY.

**0 = none or did not occur    1 = somewhat severe    2 = moderately severe    3 = extremely severe**

1. Misplacing or losing things	0	1	2	3
2. Troublesome neighbours	0	1	2	3
3. Social obligations	0	1	2	3
4. Inconsiderate smokers	0	1	2	3
5. Troubling thoughts about your future	0	1	2	3
6. Thoughts about death	0	1	2	3
7. Health of a family member	0	1	2	3
8. Not enough money for clothing	0	1	2	3
9. Not enough money for housing	0	1	2	3
10. Concerns about owing money	0	1	2	3
11. Concerns about getting credit	0	1	2	3
12. Concerns about money for emergencies	0	1	2	3
13. Someone owes you money	0	1	2	3
14. Financial responsibility for someone who does not live with you	0	1	2	3
15. Cutting down on electricity, water etc	0	1	2	3
16. Smoking too much	0	1	2	3
17. Use of alcohol	0	1	2	3
18. Personal use of drugs	0	1	2	3
19. Too many responsibilities	0	1	2	3
20. Decisions about having children	0	1	2	3
21. Non-family members living in your house	0	1	2	3
22. Care for pet	0	1	2	3
23. Planning meals	0	1	2	3
24. Concerned about the meaning of life	0	1	2	3
25. Trouble relaxing	0	1	2	3
26. Trouble making decisions	0	1	2	3
27. Problems getting along with fellow workers	0	1	2	3
28. Customers or clients giving you a hard time	0	1	2	3
29. Home maintenance (inside)	0	1	2	3
30. Concerns about job security	0	1	2	3
31. Concerns about retirement	0	1	2	3
32. Laid off or out of work	0	1	2	3
33. Don't like current work duties	0	1	2	3
34. Don't like fellow workers	0	1	2	3
35. Not enough money for basic necessities	0	1	2	3
36. Not enough money for food	0	1	2	3
37. Too many interruptions	0	1	2	3
38. Unexpected company	0	1	2	3
39. Too much time on hands	0	1	2	3
40. Having to wait	0	1	2	3
41. Concerns about accidents	0	1	2	3
42. Being lonely	0	1	2	3
43. Not enough money for healthcare	0	1	2	3
44. Fear of confrontation	0	1	2	3
45. Financial security	0	1	2	3

46. Silly practical mistakes	0	1	2	3
47. Inability to express yourself	0	1	2	3
48. Physical illness	0	1	2	3
49. Side effects of medication	0	1	2	3
50. Concerns about medical treatment	0	1	2	3
51. Physical appearance	0	1	2	3
52. Fear of rejection	0	1	2	3
53. Difficulties with getting pregnant	0	1	2	3
54. Sexual problems that results from physical problems	0	1	2	3
55. Sexual problems other than those resulting from physical problems	0	1	2	3
56. Concerns about health in general	0	1	2	3
57. Not seeing enough people	0	1	2	3
58. Friends or relatives too far away	0	1	2	3
59. Preparing meals	0	1	2	3
60. Wasting time	0	1	2	3
61. Car maintenance	0	1	2	3
62. Filling out forms	0	1	2	3
63. Neighbourhood deterioration	0	1	2	3
64. Financing children's education	0	1	2	3
65. Problems with employees	0	1	2	3
66. Problems on job due to being a man or a woman	0	1	2	3
67. Declining physical abilities	0	1	2	3
68. Being exploited	0	1	2	3
69. Concerns about bodily functions	0	1	2	3
70. Rising prices of common goods	0	1	2	3
71. Not getting enough rest	0	1	2	3
72. Not getting enough sleep	0	1	2	3
73. Problems with ageing parents	0	1	2	3
74. Problems with your children	0	1	2	3
75. Problems with persons younger than yourself	0	1	2	3
76. Problems with your lover	0	1	2	3
77. Difficulties seeing or hearing	0	1	2	3
78. Overloaded with family responsibilities	0	1	2	3
79. Too many things to do	0	1	2	3
80. Unchallenging work	0	1	2	3
81. Concerns about meeting high standards	0	1	2	3
82. Financial dealings with friends or acquaintances	0	1	2	3
83. Job dissatisfaction	0	1	2	3
84. Worries about decisions to change jobs	0	1	2	3
85. Trouble with reading, writing, or spelling abilities	0	1	2	3
86. Too many meetings	0	1	2	3
87. Problems with divorce	0	1	2	3
88. Trouble with arithmetic	0	1	2	3
89. Gossip	0	1	2	3
90. Legal problems	0	1	2	3
91. Concerns about weight	0	1	2	3
92. Not enough time to do the things you need to do	0	1	2	3
93. Television	0	1	2	3
94. Not enough personal energy	0	1	2	3
95. Concerns about inner conflicts	0	1	2	3
96. Feel conflicted over what to do	0	1	2	3
97. Regrets over past decisions	0	1	2	3
98. Menstrual (period) problems	0	1	2	3
99. The weather	0	1	2	3
100. Nightmares	0	1	2	3
101. Concerns about getting ahead	0	1	2	3

102. Hassles from boss or supervisor	0	1	2	3
103. Difficulties with friends	0	1	2	3
104. Not enough time for family	0	1	2	3
105. Transportation problems	0	1	2	3
106. Not enough money for transportation	0	1	2	3
107. Not enough money for entertainment and recreation	0	1	2	3
108. Shopping	0	1	2	3
109. Prejudice and discrimination from others	0	1	2	3
110. Property, investments or taxes	0	1	2	3
111. Not enough time for entertainment and recreation	0	1	2	3
112. Yard work or outside home maintenance	0	1	2	3
113. Concerns about news	0	1	2	3
114. Noise	0	1	2	3
115. Crime	0	1	2	3
116. Traffic	0	1	2	3
117. Pollution	0	1	2	3

How was your day today? If anything particularly unusual or stressful occurred today or if today differed from any normal day then we would like you to tell us about it!

Please detail in the space below:

---



---



---



---



---



---



---



---



---



---



---



---



---



---



---



---

Thank you

**Appendix 16: Auditory Verbal Learning Task (List of words)**

<b>List A</b>	<b>List B</b>
Drum	Desk
Curtain	Ranger
Bell	Bird
Coffee	Shoe
School	Stove
Parent	Mountain
Moon	Glasses
Garden	Towel
Hat	Cloud
Farmer	Boat
Nose	Lamb
Turkey	Gun
Colour	Pencil
House	Church
River	Fish



Cortisol Index	Effect of		
	WHR	Gender	WHR*Gender
Mean Increase	(F (1, 78) =1.612; p=0.208; NS)	(F (1, 78) =0.100; p=0.753; NS)	(F (1, 78) =0.289; p=0.592; NS)
AUC	(F (1, 78) =1.456 p=0.231; NS)	(F (1, 78) =2.386; p=0.126; NS)	(F (1, 78) =0.388; p=0.535; NS)
AURC	(F (1, 78) =1.806; p=0.183; NS)	(F (1, 78) =0.180; p=0.672; NS)	(F (1, 78) =0.221; p=0.639; NS)
Change 0-30	(F (1, 78) =2.243; p=0.138; NS)	(F (1, 78) =490; p=0.486; NS)	(F (1, 78) =0.077; p=0.782; NS)
Day Difference	(F (1, 78) =0.384; p=0.537; NS)	(F (1, 78) =0.407; p=0.526; NS)	(F (1, 78) =0.067; p=0.796; NS)
Day Difference 3-12	(F (1, 78) =0.414; p=0.522; NS)	<b>(F (1, 78) =7.596; p&lt;0.01)</b>	(F (1, 78) =0.610; p=0.437; NS)
Sample H	(F (1, 78) =0.227; p=0.635; NS)	<b>(F (1, 78) =3.963; p&lt;0.05)</b>	(F (1, 78) =0.080; p=0.779; NS)
Day Mean	<b>(F (1, 78) =4.223; p&lt;0.05)</b>	(F (1, 78) =1.584; p=0.212; NS)	(F (1, 78) =0.155; p=0.695; NS)
Diurnal Mean	<b>(F (1, 78) =2.795; p=0.099)</b>	(F (1, 78) =0.760; p=0.386; NS)	(F (1, 78) =0.541; p=0.464; NS)

Appendix 17: Table of F-values for the Cortisol Indices (Chapter Five)

**Appendix 18: State Trait Anxiety Inventory (STAI)**

Questionnaire 2      Ppt ID: EX2NL: \_\_\_      Time: \_\_:\_\_      Date: \_\_/\_\_/\_\_

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you feel right now, that is, at this moment. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

	Not at all	Somewhat	Moderately So	Very Much So
1. I feel calm	1	2	3	4
2. I feel secure	1	2	3	4
3. I am tense	1	2	3	4
4. I feel strained	1	2	3	4
5. I feel at ease	1	2	3	4
6. I feel upset	1	2	3	4
7. I am presently worried over possible misfortunes	1	2	3	4
8. I feel satisfied	1	2	3	4
9. I feel frightened	1	2	3	4
10. I feel comfortable	1	2	3	4
11. I feel self-confident	1	2	3	4
12. I feel nervous	1	2	3	4
13. I am jittery	1	2	3	4
14. I feel indecisive	1	2	3	4
15. I am relaxed	1	2	3	4
16. I feel content	1	2	3	4
17. I am worried	1	2	3	4
18. I feel confused	1	2	3	4
19. I feel steady	1	2	3	4
20. I feel pleasant	1	2	3	4

**Appendix 19: State Self Esteem Scale (SSES)**

Questionnaire 1

Ppt ID: EX2NL: \_\_\_

Time: \_\_: \_\_

Date: \_\_/\_\_/\_\_

This is a questionnaire designed to measure what you are thinking at this moment. There is, of course, no right or wrong answer for any statement. Read each statement and then circle the appropriate number to the right of the statement. The best answer is what you feel is true of yourself *at this moment*. Be sure to answer all of the items, even if you are not certain of the best answer. Again, answer these questions as they are true for you RIGHT NOW.

	Not at all	A little bit	Some- What	Very Much	Extremely
1. I feel confident about my abilities.	1	2	3	4	5
2. I am worried about whether I am regarded as a success or failure.	1	2	3	4	5
3. I feel satisfied with the way my body looks right now.	1	2	3	4	5
4. I feel frustrated or rattled about my performance.	1	2	3	4	5
5. I feel that I am having trouble understanding things that I read.	1	2	3	4	5
6. I feel that others respect and admire me.	1	2	3	4	5
7. I am dissatisfied with my weight.	1	2	3	4	5
8. I feel self-conscious.	1	2	3	4	5
9. I feel as smart as others.	1	2	3	4	5
10. I feel displeased with myself.	1	2	3	4	5
11. I feel good about myself.	1	2	3	4	5
12. I am pleased with my appearance right now.	1	2	3	4	5
13. I am worried about what other people think of me.	1	2	3	4	5
14. I feel confident that I understand things.	1	2	3	4	5
15. I feel inferior to others at this moment.	1	2	3	4	5
16. I feel unattractive.	1	2	3	4	5
17. I feel concerned about the impression I am making.	1	2	3	4	5
18. I feel that I have less scholastic ability right now than others.	1	2	3	4	5
19. I feel like I'm not doing well.	1	2	3	4	5
20. I am worried about looking foolish.	1	2	3	4	5

Task	Effect of					Condition*WHR *Gender	
	Condition	WHR	Gender	Condition*WHR	Condition*Gender		WHR*Gender
DMS: %Correct Delays	F(1, 60)=1.624; p=0.207	F(1, 60)=0.708; p=0.403	<b>F(1, 60)=3.353;</b> p=0.072	F(1, 60)=0.587; p=0.447	F(1, 60)=1.048; p=0.310	F(1, 60)=0.235; p=0.650	F(1, 60)=0.651; p=0.423
DMS: %Correct Simultaneously	F(1, 60)=0.294; p=0.590	F(1, 60)=0.262; p=0.611	F(1, 60)=0.066; p=0.798	F(1, 60)=0.468; p=0.497	F(1, 60)=0.209; p=0.649	F(1, 60)=2.447; p=0.123	F(1, 60)=0.175; p=0.679
DMS: Error Probability	F(1, 60)=0.063; p=0.803	F(1, 60)=0.117; p=0.734	F(1, 60)=0.856 p=0.359	F(1, 60)=0.036; p=0.856	F(1, 60)=2.091; p=0.153	F(1, 60)=0.373; p=0.543	<b>F(1, 60)=4.581;</b> p<0.05
PAL	F(1, 60)=2.159; p=0.147	F(1, 60)=2.018; p=0.161	F(1, 60)=0.264; p=0.609	<b>F(1, 60)=6.194;</b> p<0.05	F(1, 60)=0.894; p=0.348	F(1, 60)=0.108; p=0.744	F(1, 60)=0.708; p=0.403
PRM	F(1, 60)=0.143; p=0.707	F(1, 60)=0.803; p=0.374	<b>F(1, 60)=4.605;</b> p<0.05	F(1, 60)=1.308 p=0.257	F(1, 60)=0.118; p=0.732	F(1, 60)=0.004; p=0.950	F(1, 60)=2.695; p=0.106
RVP	F(1, 60)=0.008; p=0.929	F(1, 60)=0.000; p=0.987	F(1, 60)=0.431; p=0.514	F(1, 60)=1.332; p=0.253	F(1, 60)=3.408; p=0.070	F(1, 60)=0.088; p=0.768	F(1, 60)=0.167; p=0.684
SRM	F(1, 60)=1.563; p=0.216	<b>F(1, 60)=3.376;</b> p=0.071	<b>F(1, 60)=5.761;</b> p<0.05	F(1, 60)=0.083; p=0.774	F(1, 60)=0.001 p=0.978	F(1, 60)=0.494; p=0.485	<b>F(1, 60)=3.556;</b> p=0.064
SWM: Number of Errors	F(1, 60)=0.412; p=0.523	F(1, 60)=0.228; p=0.635	F(1, 60)=0.908; p=0.344	<b>F(1, 60)=4.279;</b> p<0.05	F(1, 60)=0.253; p=0.617	F(1, 60)=2.207; p=0.160	F(1, 60)=0.430; p=0.514
SWM: Strategy	F(1, 60)=0.294; p=0.590	F(1, 60)=0.019; p=0.891	F(1, 60)=0.706; p=0.404	F(1, 60)=2.746; p=0.103	F(1, 60)=0.473; p=0.494	F(1, 60)=0.258; p=0.617	F(1, 60)=0.347; p=0.558
SOC: Minimum Number of Moves	F(1, 60)=1.278; p=0.263	F(1, 60)=1.079; p=0.303	<b>F(1, 60)=15.421;</b> p<0.01	F(1, 60)=1.172; p=0.283	F(1, 60)=0.077; p=0.783	F(1, 60)=0.052; p=0.820	F(1, 60)=0.049; p=0.825
SOC: Mean Initial Thinking Time	F(1, 60)=1.190; p=0.172	F(1, 60)=0.296; p=0.588	F(1, 60)=1.793; p=0.186	F(1, 60)=0.064; p=0.801	F(1, 60)=0.037; p=0.848	F(1, 60)=0.185; p=0.668	F(1, 60)=0.175; p=0.677
SOC: Mean Subs. thinking time	F(1, 60)=0.452; p=0.504	F(1, 60)=0.077; p=0.782	F(1, 60)=2.574; p=0.114	F(1, 60)=2.503; p=0.119	F(1, 60)=0.000; p=0.982	F(1, 60)=0.056; p=0.814	<b>F(1, 60)=3.993;</b> p=0.05

Appendix 20: Table of F-Values for Cognitive Performance by Condition

Task	Effect of						
	Cortisol Response Group	WHR	Gender	Cortisol Response*WHR	Cortisol Response*Gender	WHR*Gender	Cortisol Response *WHR*Gender
DMS: %Correct All Delays	F(1, 51)=0.029; p=0.866	F(1, 51)=2.103; p=0.153	F(1, 51)=1.596; p=0.212	F(1, 51)=0.362; p=0.550	F(1, 51)=1.732; p=0.194	F(1, 51)=0.033; p=0.858	F(1, 51)=2.399; p=0.128
DMS: %Correct Simultaneously	F(1, 51)=0.033; p=0.856	F(1, 51)=0.321; p=0.574	F(1, 51)=0.203; p=0.654	F(1, 51)=0.025; p=0.876	F(1, 51)=0.001; p=0.979	F(1, 51)=1.613; p=0.210	F(1, 51)=0.010; p=0.921
DMS: Error Probability	F(1, 51)=0.279; p=0.600	F(1, 51)=0.108; p=0.744	F(1, 51)=0.314; p=0.578	F(1, 51)=0.004; p=0.948	F(1, 51)=2.343; p=0.132	F(1, 51)=0.277; p=0.601	<b>F(1, 51)=3.098;</b> p=0.084
PAL	F(1, 51)=0.173; p=0.679	F(1, 51)=0.270; p=0.605	F(1, 51)=0.001; p=0.974	F(1, 51)=0.284; p=0.579	F(1, 51)=1.293; p=0.261	F(1, 51)=0.044; p=0.510	F(1, 51)=2.663; p=0.109
PRM	F(1, 51)=0.056; p=0.814	F(1, 51)=0.353; p=0.555	F(1, 51)=2.509; p=0.119	F(1, 51)=0.312; p=0.579	F(1, 51)=1.002; p=0.321	F(1, 51)=0.026; p=0.873	F(1, 51)=0.795; p=0.377
RVP	F(1, 51)=0.223; p=0.639	F(1, 51)=0.141; p=0.709	F(1, 51)=0.000; p=0.985	F(1, 51)=0.217; p=0.643	F(1, 51)=0.122; p=0.728	F(1, 51)=0.055; p=0.815	F(1, 51)=0.774; p=0.383
SRM	F(1, 51)=0.012; p=0.913	F(1, 51)=1.760; p=0.190	<b>F(1, 51)=4.822;</b> p<0.05	F(1, 51)=0.179; p=0.674	F(1, 51)=0.294; p=0.590	F(1, 51)=0.180; p=0.673	<b>F(1, 51)=6.403;</b> p<0.05
SWM: Number of Errors	F(1, 51)=1.108; p=0.298	F(1, 51)=0.684; p=0.412	F(1, 51)=0.855; p=0.360	F(1, 51)=0.321; p=0.573	<b>F(1, 51)=3.055;</b> p=0.087	F(1, 51)=2.016; p=0.162	F(1, 51)=0.098; p=0.756
SWM: Strategy	F(1, 51)=0.231; p=0.633	F(1, 51)=0.061; p=0.806	F(1, 51)=1.147; p=0.289	F(1, 51)=0.187; p=0.667	F(1, 51)=2.608; p=0.113	F(1, 51)=0.697; p=0.408	F(1, 51)=0.478; p=0.492
SOC: Minimum Number of Moves	F(1, 51)=0.080; p=0.778	F(1, 51)=1.608; p=0.211	<b>F(1, 51)=8.056;</b> p<0.01	F(1, 51)=0.281; p=0.598	F(1, 51)=0.232; p=0.632	F(1, 51)=0.123; p=0.727	F(1, 51)=0.886; p=0.351
SOC: Mean Initial Thinking Time	F(1, 51)=0.043; p=0.837	F(1, 51)=0.030; p=0.863	F(1, 51)=2.014; p=0.162	F(1, 51)=0.969; p=0.330	F(1, 51)=0.276; p=0.602	F(1, 51)=0.012; p=0.912	F(1, 51)=0.107; p=0.745
SOC: Mean Subsequent thinking time	F(1, 51)=0.035; p=0.853	F(1, 51)=0.034; p=0.854	<b>F(1, 51)=2.874;</b> p=0.096	F(1, 51)=0.603; p=0.441	F(1, 51)=0.008; p=0.928	F(1, 51)=0.195; p=0.661	<b>F(1, 51)=2.096;</b> p=0.094

Appendix 21: (a) Table of F-Values for Cognitive Performance by Response Group (Cortisol)

Task	Stress Response Group	WHR	Gender	Effect of			WHR*Gender	Stress Response *WHR*Gender
				Stress Response*WHR	Stress Response*Gender	WHR*Gender		
DMS: %Correct All Delays	F(1, 51)=1.077; p=0.304	F(1, 51)=1.661; p=0.203	F(1, 51)=1.060; p=0.308	F(1, 51)=1.680; p=0.201	F(1, 51)=1.486; p=0.228	F(1, 51)=0.136; p=0.714	F(1, 51)=0.159; p=0.692	
DMS: %Correct Simultaneously	F(1, 51)=0.187; p=0.667	F(1, 51)=0.183; p=0.670	F(1, 51)=0.367; p=0.547	F(1, 51)=0.939; p=0.337	F(1, 51)=1.074; p=0.305	F(1, 51)=2.237; p=0.141	F(1, 51)=0.048; p=0.827	
DMS: Error Probability	F(1, 51)=0.063; p=0.803	F(1, 51)=0.029; p=0.864	F(1, 51)=0.005; p=0.945	F(1, 51)=0.002; p=0.963	F(1, 51)=2.987; p=0.090	F(1, 51)=1.188; p=0.281	F(1, 51)=4.870; p<0.05	
PAL	F(1, 51)=3.243; p=0.078	F(1, 51)=0.478; p=0.492	F(1, 51)=0.037; p=0.849	F(1, 51)=3.970; p=0.052	F(1, 51)=0.053; p=0.819	F(1, 51)=1.143; p=0.290	F(1, 51)=0.510; p=0.479	
PRM	F(1, 51)=0.090; p=0.765	F(1, 51)=0.730; p=0.397	F(1, 51)=2.571; p=0.115	F(1, 51)=0.122; p=0.729	F(1, 51)=1.044; p=0.312	F(1, 51)=0.161; p=0.690	F(1, 51)=1.645; p=0.205	
RVP	F(1, 51)=0.362; p=0.550	F(1, 51)=0.170; p=0.682	F(1, 51)=0.005; p=0.944	F(1, 51)=2.068; p=0.155	F(1, 51)=0.380; p=0.540	F(1, 51)=0.246; p=0.622	F(1, 51)=0.001; p=0.974	
SRM	F(1, 51)=0.232; p=0.632	F(1, 51)=3.330; p=0.074	F(1, 51)=3.528; p=0.066	F(1, 51)=0.107; p=0.745	F(1, 51)=0.013; p=0.911	F(1, 51)=1.410; p=0.241	F(1, 51)=4.841; p<0.05	
SWM: Number of Errors	F(1, 51)=0.049; p=0.826	F(1, 51)=0.468; p=0.497	F(1, 51)=0.369; p=0.546	F(1, 51)=2.046; p=0.159	F(1, 51)=2.036; p=0.160	F(1, 51)=2.548; p=0.117	F(1, 51)=2.944; p=0.092	
SWM: Strategy	F(1, 51)=0.052; p=0.821	F(1, 51)=0.001; p=0.974	F(1, 51)=0.857; p=0.359	F(1, 51)=0.461; p=0.500	F(1, 51)=1.975; p=0.166	F(1, 51)=1.184; p=0.282	F(1, 51)=2.242; p=0.140	
SOC: Minimum Number of Moves	F(1, 51)=1.370; p=0.247	F(1, 51)=1.395; p=0.243	F(1, 51)=9.364; p<0.01	F(1, 51)=0.526; p=0.472	F(1, 51)=0.041; p=0.841	F(1, 51)=0.170; p=0.682	F(1, 51)=0.124; p=0.727	
SOC: Mean Initial Thinking Time	F(1, 51)=0.069; p=0.793	F(1, 51)=0.001; p=0.982	F(1, 51)=1.280; p=0.263	F(1, 51)=0.021; p=0.886	F(1, 51)=0.176; p=0.677	F(1, 51)=0.074; p=0.787	F(1, 51)=3.929; p=0.053	
SOC: Mean Subsequent thinking time	F(1, 51)=0.253; p=0.617	F(1, 51)=0.103; p=0.750	F(1, 51)=3.271; p=0.076	F(1, 51)=3.286; p=0.076	F(1, 51)=0.264; p=0.610	F(1, 51)=0.013; p=0.909	F(1, 51)=0.842; p=0.363	

(b) Table of F-Values for Cognitive Performance by Response Group (Cortisol and Blood Pressure)

**Appendix 22: Cortisol secreted across the test session from alternative baseline in High/Low WHR Males/Females (Means  $\pm$  SEM)**

