RELATIONSHIPS BETWEEN BODY MASS INDEX, APPETITE REGULATION & PHYSICAL ACTIVITY DURING SHIFT-WORK & NIGHT-WORK

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The following Figures have been omitted on request of the University –

Fig. 2.1 pg. 7

Fig. 2.2 pg. 9

Fig. 2.3 pg. 13

Fig. 2.4 pg. 17

Fig. 2.5 pg. 19

Fig. 2.7 pg. 26

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ABSTRACT

Approximately 3.6 million individuals in the UK are involved in a type of shift-work which impinges on the normal nocturnal sleeping period. This prevalence has significance considering that shift-work is a risk factor for many health problems including cardiovascular disease, breast cancer, metabolic syndrome, obesity and gastrointestinal symptoms (e.g., constipation). These health inequalities are generally under-researched. Past studies have also focused on chronobiological-related reasons (e.g., exposure to light at night) rather than lifestyle factors. Physical activity is reported to be beneficial for many aspects of day-worker's health. However, there is a dearth of knowledge regarding the relationship between physical activity, adiposity and gastrointestinal symptoms in shift-workers. Thus, the aim of this thesis was to explore, using a multidisciplinary approach, the relationships between body mass index (BMI), appetite regulation, gastrointestinal health and physical activity during shift-work and night-work.

In the first study (presented in Chapter 3), a cross-sectional study design was adopted to explore the relationships between BMI, gastrointestinal symptoms and leisure-time physical activity (LTPA) in shift-workers. The data from this study indicated that the least active shift-workers had the highest mean BMI, 73% of these workers being overweight or obese. Nevertheless, dose-response effects of LTPA on BMI were not evident. A positive relationship was present between physical activity level and frequency of heartburn but not other digestive symptoms (e.g., diarrhoea).

In the studies presented in Chapters 4 and 5, the effects of an acute bout of evening exercise upon appetite-related factors were investigated during a simulated night-shift. In Chapter 4, the protocol was characterised by a feeding schedule typically adopted by many shift-workers, i.e. eating smaller but more frequent

portions of food rather than one large meal. In contrast, the protocol in Chapter 5 was characterised by a feeding schedule designed to be more in line with what day-workers do during their work-period, i.e. eating one larger meal 3-4 h after the work period has started. Findings from Chapter 4 indicate that, unlike after diurnal exercise, circulating concentrations of acylated ghrelin and leptin during a night-shift are increased by prior evening exercise. However, hunger during the night-shift was unaffected by prior evening exercise in this study. In the subsequent experiment involving one large meal, nocturnal concentration of serum leptin was increased by exercise but there was little effect on plasma acylated ghrelin level. Again, night-shift hunger was unaffected by prior evening exercise. Taken together, these findings indicated that exercise mediates different effects on appetite-related hormones at night and that meal frequency is an important factor which regulates the response of acylated ghrelin, but not leptin. Despite the above findings, mean night-shift hunger was unaffected by evening exercise, regardless of meal frequency. This implies that a compensatory increase in food intake during the night-shift in response to prior exercise may not occur, thus supporting the notion that exercise can mediate a negative energy balance which might attenuate body mass gain in shift-workers.

The study presented in Chapter 6 determined the within-subject correlations between factors that regulate appetite in the post-exercise period. This study explored how circulating levels of acylated ghrelin and leptin are controlled at night following evening exercise. The findings from Chapter 6 suggested that exercise-related changes in plasma acylated ghrelin concentration are negatively correlated to those in circulating levels of glucose and insulin, but not those in non-esterified fatty acids (NEFA) or triglyceride. The aforementioned significant correlations were not reported in daytime studies. Post-exercise alterations in serum leptin level were also

found to be related to those in circulating levels of insulin but not those in glucose, NEFA or triglyceride. The exercise-related alterations in circulating level of acylated ghrelin, but not leptin were correlated with the changes in hunger during the post-exercise period throughout the night.

In the study presented in Chapter 7, a randomised controlled trial was employed to examine the effect of altering (via motivational interviewing over a three-month period) a shift-worker's physical activity and dietary habits on their adiposity and gastrointestinal symptoms. The findings presented in Chapter 7 indicate that a 12-week motivational interviewing intervention which focused upon increasing physical activity level and improving dietary habits significantly attenuated an increase in BMI, but not waist-to-hip ratio or frequency of digestive symptoms in UK shift-workers.

In summary, this thesis makes a significant contribution to the field of physical activity and shift-work. This thesis demonstrates that relationships between BMI, appetite regulation, gastrointestinal health (i.e., heartburn) and physical activity do exist during shift-work and night-work.

DECLARATIONS

I declare that work presented in this thesis is entirely my own. Some of the work has been presented at international conferences and has been accepted for full publication:

Morris C, Fullick S, Grindey C, Waterhouse J, Atkinson G. Leisure-time physical activity reduces self-reported gastrointestinal problems of shift-workers. European College of Sports Sciences – Estoril 2008.

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TABLE OF CONTENTS

TITLE PAGE i

ACKNOWLEDGEMENTS ii

ABSTRACT iii

DECLARATIONS vi

TABLE OF CONTENTS vii

LIST OF FIGURES xiii

LIST OF TABLES xvi

ABBREVIATIONS xviii

CHAPTER 1: INTRODUCTION 1

1.1 Introduction to the National Prevention Research Initiative 2

1.2 Statement of the problem 2

1.3 Aims 4

1.4 Objectives 4

CHAPTER 2: REVIEW OF LITERATURE 5

2.1 Introduction to shift-work 6

2.1.1 Prevalence of shift-work 6

2.1.2 Gender and prevalence of shift-work 7

2.1.3 Age and shift-work prevalence 8

2.1.4 Shift-work prevalence in industries 9

2.2 Types of shift-work 9

2.2.1 Two-shift system 10

2.2.2 Three-shift system 10

2.2.3 Continental-shifts 10

2.2.4 Split-shifts 10

2.2.5 Permanent shifts 11

2.3 Epidemiology of shift-work 11

2.3.1 Shift-work and excess adiposity 11
2.3.2 Shift-work and gastrointestinal health 13
2.4 Mechanisms underlying an adverse effect of shift-work on adiposity and gastrointestinal health 14
2.4.1 Human rhythms 15
2.4.2 Adiposity: desynchronized biological/behavioral rhythms, psychological and lifestyle factors 15
2.4.2.1 Desynchronisation and regulators of energy intake 16
2.4.2.1.1 Ghrelin and leptin 16
2.4.2.1.2 Desynchronisation, ghrelin and leptin 18
2.4.2.2 Shift-work and psychological disorders 20
2.4.2.3 Shift-work and energy intake 22
2.4.2.4 Shift-work and physical activity habits 23
2.4.2.5 Temporal position of food intake and adiposity 24
2.4.2.6 Summary of possible mechanism underlying the adverse association between shift-work and adiposity 26
2.4.3 Shift-work and gastrointestinal symptoms 27
2.4.3.1 Summary of possible mechanisms underlying the adverse association between shift-work and gastrointestinal health 28
2.5 Physical activity and adiposity 28
2.5.1 Physical activity and primary prevention of body mass gain in day-workers 29
2.5.2 Physical activity for body mass reduction in day-workers 30
2.5.3 Physical activity for maintenance of reduced body mass in day-workers 30
2.5.4 Physical activity and adiposity in shift-workers 31
2.5.5 Summary of relationship between physical activity and adiposity 32
2.6 Mechanisms underlying favorable effects of physical activity on adiposity 32
2.6.1 Physical activity and energy expenditure 33
2.6.2 Physical activity and energy intake 34
2.6.2.1 Physical activity and ghrelin 34
2.6.2.2 Physical activity and leptin 36
2.6.2.3 Physical activity, hunger and food intake 37

2.6.3 Summary of mechanisms underlying favorable effects of physical activity on adiposity 39
2.7 Physical activity and gastrointestinal health 39
2.7.1 Physical activity and gastrointestinal health in shift-workers 41
2.7.2 Summary of physical activity and gastrointestinal health 41
2.8 Synopsis 41

CHAPTER 3: RELATIONSHIPS BETWEEN LEISURE-TIME PHYSICAL ACTIVITY, BODY MASS INDEX AND DIGESTIVE SYMPTOMS IN SHIFT-WORKERS 43

3.1 Introduction 44
3.2 Methods 46
3.2.1 Study population 46
3.2.2 Leisure-time physical activity assessment 48
3.2.3 Body mass index assessment 48
3.2.4 Digestive health assessment 48
3.2.5 Assessment of confounders 49
3.2.5 Statistical analysis 49
3.3 Results 50
3.3.1 Body mass index and leisure-time physical activity 50
3.3.2 Digestive health and leisure-time physical activity 51
3.4 Discussion 53
3.5 Conclusion 56

CHAPTER 4: ACUTE EFFECTS OF EVENING EXERCISE ON APPETITE RELATED FACTORS DURING A SIMULATED NIGHT-SHIFT WHILST A 'TYPICAL' FEEDING SCHEDULE IS USED 58

4.1 Introduction 59
4.2 Methods 60
4.2.1 Participants 60
4.2.2 Preliminary measurements 61
4.2.3 Experimental procedures 61

4.2.4 Test meals 63
4.2.5 Perceived hunger and activity 63
4.2.6 Blood sampling 63
4.2.7 Biochemistry 65
4.2.8 Statistical analysis 65
4.3 Results 66
4.4 Discussion 69
4.5 Conclusion 74

CHAPTER 5: ACUTE EFFECTS OF EVENING EXERCISE ON APPETITE RELATED FACTORS DURING A SIMULATED NIGHT-SHIFT WHILST AN 'ATYPICAL' FEEDING SCHEDULE IS USED 75

5.1 Introduction 76
5.2 Methods 77
5.2.1 Participants 77
5.2.2 Preliminary measurements 77
5.2.3 Experimental procedures 77
5.2.4 Test meals 78
5.2.5 Perceived hunger and activity 78
5.2.6 Blood sampling 78
5.2.7 Biochemistry 78
5.2.8 Statistical analysis 79
5.3 Results 79
5.4 Discussion 83
5.5 Conclusion 86

CHAPTER 6: WITHIN-SUBJECT RELATIONSHIPS BETWEEN POST-EXERCISE CONCENTRATIONS OF ACYLATED GHRELIN, LEPTIN AND OTHER VARIBLES RELATED TO HUMAN METABOLISM 88

6.1 Introduction 89
6.2 Methods 90
6.2.1 Participants 90

6.2.2 Preliminary measurements 90
6.2.3 Experimental procedures 90
6.2.4 Test meals 91
6.2.5 Perceived hunger 91
6.2.6 Blood sampling 91
6.2.7 Biochemistry 91
6.2.8 Statistical analysis 91
6.3 Results 92
6.3.1 Acylated ghrelin 92
6.3.2 Leptin 94
6.4 Discussion 95
6.5 Conclusion 98

CHAPTER 7: EFFECTS OF A LIFESTYLE INTERVENTION BASED ON MOTIVATIONAL INTERVIEWING ON SHIFT-WORKER'S ADIPOSITY AND DIGESTIVE HEALTH: A RANDOMISED CONTROLLED TRIAL 99

7.1 Introduction 100
7.2 Methods 102
7.2.1 Participants and setting 102
7.2.2 Study design 102
7.2.3 Intervention group – motivational interviewing 103
7.2.4 Comparator group 104
7.2.5 Measurement protocol 104
7.2.6 Physical activity questionnaire 105
7.2.7 Digestive health questionnaire 106
7.2.8 Food frequency questionnaire 106
7.2.9 Statistical analysis 107
7.3 Results 108
7.4 Discussion 112
7.5 Conclusion 116

CHAPTER 8: SYNTHESIS OF FINDINGS 118

8.1 Introduction 119
8.2 Realisation of objectives 119
8.3 General discussion 122
8.3.1 Chapter 3: Relationships between leisure-time physical activity, body mass index and digestive symptoms in shift-workers 122
8.3.2 Chapter 4: Acute effects of evening exercise on appetite related factors during a simulated night-shift whilst a 'typical' feeding schedules is used 124
8.3.3 Chapter 5: Acute effects of evening exercise on appetite related factors during a simulated night-shift whilst an 'atypical' feeding schedules is used 126
8.3.4 Chapter 6: Within-subject relationships between post-exercise concentrations of acylated ghrelin, leptin and other variables related to human metabolism 129
8.3.5 Chapter 7: Effects of a lifestyle intervention based on motivational interviewing on shift-worker's adiposity and digestive health: a randomised controlled trial 131
8.3.6 Conclusion 133
8.3.7 Future directions 134

REFERENCES 136

LIST OF FIGURES

Figure 2.1. Proportions of employees who never under undertook shift-work in the UK between 1993 and 2003 (adapted from McOrmond, 2004) 7

Figure 2.2. Proportions of employees that undertake shift-work in Europe, effect of age (adapted from Boisard et al., 2003) 9

Figure 2.3. The trend of percentage BMI increase relative to BMI at entry in participants who were followed for 14 years (Suwazono et al., 2008) 13

Figure 2.4. Effect of intravenous infusion of ghrelin on energy intake from a buffet. *** indicates significant ($P<0.05$) difference between conditions (Wren et al., 2001a) 17

Figure 2.5. Duration of main sleep episode by groups and shifts. *$P<0.05$ with fixed daytime work schedules (Ohayon et al., 2002) 19

Figure 2.6. Patterns of energy expenditure after ingestion of a meal at different times of day for a 60 min period of measurement (Romon et al., 1993). Data are expressed as mean±SEM 26

Figure 4.1. Circulating acylated ghrelin (**A**) and leptin (**B**) concentrations and hunger (**C**) during exercise and control trials (mean±SD). *Solid rectangle, cycling; grey square, test meal consumption*..... 67

Figure 4.2. Circulating insulin (**A**), triglyceride (**B**) and glucose (**C**) concentrations during exercise and control trials (mean±SD). *Solid rectangle, cycling; grey square, test meal consumption*..... 68

Figure 5.1. Circulating acylated ghrelin (**A**) and leptin (**B**) concentrations and hunger (**C**) during exercise and control trials (mean±SD). *Solid rectangle, cycling; grey square, test meal consumption*..... 80

Figure 5.2. Circulating insulin (**A**), triglyceride (**B**) and glucose (**C**) concentrations during exercise and control trials (mean±SD). *Solid rectangle, cycling; grey square, test meal consumption*..... 82

Figure 6.1. Plasma acylated ghrelin level against serum leptin concentration, with regression lines fitted for each participants..... 92

Figure 6.2. Plasma acylated ghrelin level against plasma glucose concentration, with regression lines fitted for each participants 93

Figure 6.3. Plasma acylated ghrelin level against hunger ratings, with regression lines fitted for each participants 93

Figure 6.4. Serum leptin level against serum insulin concentration, with regression lines fitted for each participants 94

Figure 7.1. Study flow diagram 110

Figure 8.1. Comparison of serum insulin data reported in Chapters 4 (**A**) and 5 (**B**) (mean±SD). *Solid rectangle, cycling; grey square, test meal consumption*..... 128

LIST OF TABLES

Table 3.1. Characteristics of shift-workers studied 47

Table 3.2. Statistical significance (exact *P*-values) from exploration of body mass index and digestive health symptoms associations with possible confounders 50

Table 3.3. Unadjusted and adjusted associations of leisure-time physical activity with body mass index and total digestive health score 51

Table 3.4. Unadjusted odds ratios and 95% confidence intervals between leisure-time physical activity and frequency of digestive symptoms 52

Table 3.5. Adjusted odds ratios and 95% confidence intervals between leisure-time physical activity and frequency of digestive symptoms 53

Table 6.1. Within-subject correlations between plasma acylated ghrelin and other variables 94

Table 6.2. Within-subject correlations between serum leptin and other variables 95

Table 7.1 Baseline characteristics of participants 108

Table 7.2. Baseline and post-study data for the control and intervention group 112

ABBREVIATIONS

BMI	Body mass index
CV	Coefficient of variation
IPAQ	International physical activity questionnaire
LTPA	Leisure-time physical activity
NEFA	Non-esterified fatty acids
SD	Standard deviation
SEM	Standard error of the mean

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION TO THE NATIONAL PREVENTION RESEARCH INITIATIVE

The National Prevention Research Initiative (NPRI) is a focussed collaboration involving government departments, major medical charities and research councils that work together to encourage and support research into chronic disease prevention. Fundamentally the NPRI community of researchers aim to develop and implement successful, cost-effective health interventions that reduce people's risk of developing major diseases by influencing their health behaviours.

On 19th of January 2006, the Advisory Board of the National Prevention Research Initiative approved the award of a grant to the Research Institute for Sport and Exercise Sciences within Liverpool John Moores University. The award was made by the Medical Research Council on behalf of the eleven NPRI Funding Partners for a project entitled "Shift work and Health: optimal timing of meals and physical activity". Funding was provided for two research students, Sarah Fullick and the present author, to begin working collaboratively on the project on 1st June 2006. Some of the research work from the project is presented in this thesis, which concentrates on the impact of leisure-time physical activity (LTPA) on factors related to health, such as body mass index and digestive symptoms. Sarah Fullick's PhD thesis specifically focuses on other health related outcomes such as short-term coping strategies and blood pressure. Although, participants involved in the project have been included in both theses and some measurement aspects are common (e.g. the peak oxygen uptake of the participants), all formulation and examination of research questions was separate and distinct.

1.2 STATEMENT OF THE PROBLEM

Approximately 20% of the European work force is involved in some type of shift work involving periods of night work in order to meet the round-the-clock demands of society (Harrington, 2001, Rajaratnam and Arendt, 2001). Examples of industries utilising shift work include the emergency, retail and financial services, oil companies, and airlines. Shift systems typically involve working 6-12 hours per day and the worker alternating on two,

three, or four shifts in any 24 hour period (Harrington, 2001). It should be noted that some shift workers solely work during the hours of daylight (e.g. on early morning or afternoon shifts) or solely at night (so-called 'permanent night workers'), whereas others rotate through all three aforementioned shifts at varying speeds (changes every few days, every week or longer) and in clockwise or counter-clockwise directions (Harrington, 2001, Costa, 2003).

In comparison to working 'normal' daytime hours (e.g., 09:00 to 17:00 h), working shifts is beneficial in some regards (e.g., increased salary and use of gyms at off-peak times), but has been reported to be detrimental to many aspects of health (Harrington, 2001, Rajaratnam and Arendt, 2001, Costa, 2003). For example, it is now known that shift work increases the risk of cardiovascular disease (Boggild and Knutsson, 1999, Brown et al., 2009), excess adiposity (van Amelsvoort et al., 1999, Suwazono et al., 2008), digestive symptoms (Segawa et al., 1987, Zober et al., 1998, Harrington, 2001), metabolic syndrome (De Bacquer et al., 2009, Lin et al., 2009), sleep loss (Akerstedt, 2003), anxiety and depression (Bara and Arber, 2009), and fatigue (Shen et al., 2006). These findings are a cause for concern given the large number of people involved in shift work. Clearly an exploration of strategies that may reduce the negative effect of shift work on health is warranted. Such an exploration in relation to excess adiposity and digestive symptoms is the focus of the present thesis.

Physical activity has been implicated as an effective tool to prevent the development of excess adiposity (Rissanen et al., 1991, Owens et al., 1992, McTiernan et al., 2007) and reduce some digestive symptoms (Daley et al., 2008) in day workers. However, the relationship of physical activity with adiposity and digestive symptoms in shift-workers is unknown. This void in research is probably due to past work mainly concentrating on factors (e.g., appropriately timed consumption of melatonin) that encourage realignment of the 'body clock' to altered behaviour (Arendt, 1999). Such strategies are unlikely to be appropriately implemented by shift-workers.

1.3 AIMS

The aims of this thesis are (i) to explore the relationships between LTPA, adiposity and frequency of digestive symptoms in shift-workers, (ii) establish the effect an acute bout of physical activity upon appetite-related factors during a simulated night-shift and (iii) examine the effects of a lifestyle intervention on adiposity and frequency of digestive symptoms in shift-workers.

1.4 OBJECTIVES

The aims described above will be attained via the following objectives:

1. Determine, using a cross-sectional study design, the general relationships between LTPA, body mass index and digestive symptoms in shift-workers.
2. Determine, via an experimental study design, the acute effects of evening exercise on appetite-related factors during a simulated night-shift whilst a 'typical' feeding schedule is used.
3. Determine, via an experimental study design, the acute effects of evening exercise on appetite-related factors during a simulated night-shift whilst an 'atypical' feeding schedule is used.
4. Determine, using appropriate within-subjects correlation analyses, the relationships between different factors that regulate appetite in the post-exercise period.
5. Determine, using a randomised controlled trial, the effect of altering (via motivational interviewing over a three-month period) a shift-worker's physical activity and dietary habits on their adiposity and digestive symptoms.

CHAPTER 2

REVIEW OF LITRATURE

2.1 INTRODUCTION TO SHIFT-WORK

No universal definition of shift-work exists. Shift-work is considered to encompass work activity scheduled outside 'normal' daytime hours (Harrington, 2001). Day-work also lacks a clear definition, but is generally considered to consist of work activities between 07:00 and 19:00 h (Health and Safety Executive, 2006) . Some examples that help define shift-work include: (i) a handover of duty from one individual or work group to another; (ii) when an employee replaces another on the identical job in a 24-h period; (iii) working during morning, afternoon or night (outside 'normal' day-work hours); (iv) extended periods of work (>12 h) and (v) rotating hours of work (McOrmond, 2004). Although shift-work is somewhat difficult to define, the prevalence of this unusual type of employment is high.

2.1.1 Prevalence of shift-work

Society's demand for 24-h services (e.g., banking and transport) has caused the prevalence of shift-work to be high. In the US, ~15% of the workforce is involved in shift-work, with ~10% undertaking periods of night-work (US Department of Labor, 2005). In Europe, ~28% of the workforce has variable work patterns; ~10% work in the evening or at night and ~17% work either two-shift or three-shift rotating schedules (Boisard et al., 2003). Specifically in the UK, 14% of the workforce is estimated to be involved in shift-work 'most of the time' (McOrmond, 2004).

In the UK, between 1993 and 2003, the percentage of employees reporting never undertaking shift-work remained relatively stable (McOrmond, 2004; Figure 2.1). However, the proportion of employees that undertake shift-work frequently, rather than infrequently, increased by 2% between 1993 and 2003. This indicates

that the demand for permanent shift-workers is increasing, perhaps reflecting society's ever increasing thirst for services 'around the clock'.

Figure 2.1. Proportions of employees who never undertook shift-work in the UK between 1993 and 2003 (adpated from McOrmond, 2004).

2.1.2 Gender and prevalence of shift-work

A gender bias is present in prevalence of shift-work. For example, 16% of men undertake shift-work frequently compared to 13% of women (McOrmond, 2004). It is difficult to determine the exact reasons for above gender bias, but it may related to a greater proportion of men than women being employed in industries likely to utilise shift-work, such as manufacturing (McOrmond, 2004). However, it should be noted that health related occupations are predominately occupied by females and such industry also utilise shift-work (McOrmond, 2004). Notably, as UK economy shifts from manufacturing to providing services, the abovementioned gender bias may become insignificant or even reversed. Indeed, the 3% and 1% increase in women

and men, respectively, undertaking shift-work between 1993 and 2003 would support the above notion (McOrmond, 2004).

2.1.3 Age and shift-work prevalence

An age bias is also present in the prevalence of shift-work. For example, as depicted in Figure 2.2, proportion of individuals who undertake shift-work is similar between the ages of 15 to 44, however a sharp decline in the percentage of employees involved in shift-work occurs when workers are >45 years old. This may reflect the older worker's greater difficulty of adjusting to shift-work compared to a young shift-worker. Specifically, the older (>40 years), rather than younger shift-worker, experiences more sleep disruption (Matsumoto and Morita, 1987, Parkes, 1994) and has an inferior ability to readjust his or her circadian rhythms to alternating schedules (Harma et al., 1990). Interestingly, the age group, 16-19 years, regardless of gender, showed the greatest increase (8%) in involvement in shift-work between 1993 and 2003 in the UK (McOrmond, 2004). This finding may reflect young individuals needing employment which occurs around educational courses which typically take place during the day or early evening.

Figure 2.2. Proportions of employees that undertake shift-work in Europe, effect of age (adapted from Boisard et al., 2003).

2.1.4 Shift-work prevalence in industries

In the UK, industries including manufacturing, construction, transport, communication, education, health, restaurants and banking utilise shift-work (McOrmond, 2004). In 2003, shift-work was most prevalent (27%) in the transport and communication industry, regardless of age and gender (McOrmond, 2004). Shift-work was least common (~1%) in the construction industry, perhaps suggesting working at night in this industry is difficult due to the lack of sufficient light if working outside amongst other factors.

2.2 TYPES OF SHIFT-WORK

A range of shift-work schedules have been designed and are implemented in order to provide a workforce with extended working hours. Such shift-work schedules

include the two-shift and three-shift system, continental-shifts, split-shifts and permanent-shifts. These shift schedules are described briefly below.

2.2.1 Two-shift system

Two-shifts, lasting eight hours each that are normally alternated weekly or over longer intervals. An example of the two-shift system is working between 06:00 and 14:00 h for a number of days, then switching to working between 14:00 and 22:00 h.

2.2.2 Three-shift system

Morning-, afternoon- and night-shifts are used to provide a workforce which covers a 24 h period. Sometimes, individuals will work for one week on the morning-shift, followed by one week of working the afternoon-shift, followed by one week of working the night-shift. The morning-shift may occur between 06:00 and 14:00 h, the afternoon-shift sometimes takes place between 14:00 and 22:00 h and the night-shift commonly occurs between 22:00 and 06:00 h.

2.2.3 Continental-shifts

This is very similar to the three-shift system described above; however rapid rotation (forward or backwards) occurs through the three-shifts. For example, rapid forward rotation would include working two morning-shifts, then two afternoon-shifts followed by two night-shifts.

2.2.4 Split-shifts

An individual's shift is split into two segments, with a gap of several hours in between. For example, an employee may work between 05:00 and 09:00 h and then

have a rest period until 15:00 h, then work until 19:00 h. This shift system is commonly used in industries that have high workloads at two distinct periods in any given 24 h. Such industries that utilise this shift-work schedule include public transport and cleaners.

2.2.5 Permanent shifts

An employee permanently works a particular shift. For example, an individual would consistently work the night-shift.

2.3 EPIDEMIOLOGY OF SHIFT-WORK

Section 2.1 highlighted that millions of people undertake shift-work in Western societies. The prevalence of shift-work is worrying considering that this type of employment is a risk factor for many health problems including excess adiposity and digestive symptoms. The focus of this thesis is shift-work, adiposity and digestive symptoms and therefore only these factors are reviewed below. Readers interested in a general overview of the relationships between shift-work and various health problems (e.g., cardiovascular disease) should see Harrington (2001) or Costa (2003)

2.3.1 Shift-work and excess adiposity

Many researchers, using a cross-sectional study design, have reported a significant positive relationship between shift-work and measures of adiposity (Rosmond et al., 1996, van Amelsvoort et al., 1999, Parkes, 2002, Di Lorenzo et al., 2003, Karlsson et al., 2003, Ishizaki et al., 2004, Morikawa et al., 2007), whereas others have not observed such a correlation (Karlsson et al., 2001, Ha and Park, 2005a, Ostry et al.,

2006, Nabe-Nielsen et al., 2008). Some scientists, using a prospective study design, have reported that shift-work is risk factor for excess adiposity (Niedhammer et al., 1996, Morikawa et al., 2007, Suwazono et al., 2008; Figure 2.3), whereas others have reported no relationship (Watari et al., 2006, De Bacquer et al., 2009). Geliebter et al. (2000) asked day- and night-workers to recall how much body mass, if any, they had gained since working their respective schedules. Geliebter et al. (2000) reported that the night-workers had gained 3.7 kg more in body mass than the day-workers. Although the above reports provide useful data, the findings of these studies should be viewed with caution due to methodological problems. First, the investigation by Geliebter et al. (2000) relied on body mass data recalled over several years and therefore the validity of the data collected is questionable. Second, none of the abovementioned studies adequately accounted for energy expenditure or energy intake (due to the use of primitive assessments) which is surprising considering these two factors are fundamental in the regulation of adiposity. Third, a cross-sectional design does not allow causality to be determined. Fourth, cross-sectional and prospective studies require the inclusion of possible confounding variables within any analysis, which is impossible to fully achieve. Despite the abovementioned methodological concerns, the available evidence, on balance indicates that shift-work is a risk factor for excess adiposity. This is concerning considering that excess adiposity is positively related to many health problems such as cardiovascular disease, diabetes and some cancers (van Gaal et al., 2006, Zhang et al., 2008).

Figure 2.3. The trend of percentage BMI increase relative to BMI at entry in participants who were followed for 14 years (Suwazono et al., 2008).

2.3.2 Shift-work and gastrointestinal health

The relationship between shift-work and a range of digestive symptoms has been explored. Such digestive symptoms include abdominal pain, heartburn, constipation, diarrhoea, appetite disruption, stomach upsets, nauseous, indigestion, bloated stomach or flatulence. Thus, in this thesis, the term 'digestive symptoms', refers to the abovementioned gastrointestinal problems.

Some researchers, using a cross-sectional study design, have reported that the prevalence of digestive symptoms is higher in shift- than day-workers (Koller et al., 1978, Smith and Colligan, 1982, Koller, 1983, Ottmann et al., 1989), whereas others have reported no relationship (Costa et al., 1990, Poole et al., 1992, Jaffe et al., 1996). Angersbach (1980) undertook a retrospective cohort study and reported that shift-workers were significantly more likely to consult occupational health

regarding gastrointestinal problems than day-workers. Moreover, researchers have reported a positive association between shift-work and prevalence of gastrointestinal ulcers (Costa et al., 1980, Segawa et al., 1987, Tuechsen et al., 1994). Despite the above positive associations, there appears to be no relationship between shift-work and self-medication for digestive problems (Gordon et al., 1986). This perhaps indicates that shift-workers suffer from gastrointestinal discomfort in 'silence'. However, the amount of products available to treat gastrointestinal problems has increased since the 1980s and therefore it is possible that shift-workers now receive more digestive medication than day-workers. Despite many of methodological concerns discussed in section 2.3.1 also applying to the studies that explored the relationship between work schedules and gastrointestinal health, the available evidence suggests that shift-work is a risk factor for a range of gastrointestinal problems. This is worrying, considering that digestive health is inversely related to an individual's health-related quality of life (Glia and Lindberg, 1997, Revicki et al., 1998, Dennison et al., 2005).

2.4 MECHANIMS UNDERLYING ADVERSE EFFECT OF SHIFT-WORK ON ADIPOSITY AND GASTROINTESTINAL HEALTH

As reviewed in section 2.3, shift-work appears to be a risk factor for excess adiposity and gastrointestinal problems. Three pathways have been suggested to explain the association between shift-work and health problems: (i) desynchronisation of biological/behavioural rhythms, (ii) disturbed psychology and (iii) unfavourable alterations in lifestyle habits (Bøggild and Knutsson, 1999, Knutsson and Boggild, 2000). The relationship of these three pathways with excess adiposity and gastrointestinal problems is reviewed below.

2.4.1 Human rhythms

Many aspects of human behaviour and biology follow a rhythmic pattern that can be split into seasonal (e.g., platelet serotonin secretion), circadian (e.g., melatonin secretion), diurnal (e.g., core temperate) and/or ultradian (insulin secretion rate) rhythms (Wirz-Justice et al., 1977, Omeara et al., 1993, Duffy et al., 1999, Morris et al., 2009). Human rhythms are influenced by endogenous and exogenous factors. Internal biological clocks (central timekeeping output comes from suprachiasmatic nuclei), as shown using a constant routine protocol, cause rhythmic variations in many biological processes (e.g., melatonin secretion) independent of external time cues (e.g., the sleep/wake cycle). Exogenous factors are described as *zeitgebers* (time givers) and act upon internal biological clocks to mediate entrainment of cyclilic variations. Exogenous factors include light/darkness (Miyamoto and Sancar, 1998, Waterhouse et al., 2004), food consumption (Mendoza, 2007) and exercise (Buxton et al., 2003). Changing the temporal exposure (relative to biological time) of *zeitgebers*, in particular, the light/dark cycle will alter the phase of internal clocks and thus cause circadian misalignment. Fortunately, this does not occur in day-workers but does in shift-workers and individuals that travel rapidly across many time-zones and therefore these individuals are exposed to the adverse effects of internal desynchronisation.

2.4.2 Adiposity: desynchronised biological/behavioural rhythms, psychological and lifestyle factors

Excess adiposity is caused by an energy imbalance, with energy intake being greater than energy expenditure (Atkinson et al., 2008). Thus, two factors should be discussed when considering adiposity: energy intake and energy expenditure.

Factors affecting energy intake and energy expenditure in relation to shift-work are discussed below.

2.4.2.1 Desynchronisation and regulators of energy intake

Although many factors regulate energy intake (e.g., pancreatic polypeptide, peptide YY, cholecystokinin and oxyntomodulin), the focus of this thesis is on ghrelin and leptin because these two hormones have been researched considerably (Bloom et al., 2008).

2.4.2.1.1 Ghrelin and leptin

Ghrelin is a 28-ammino residue peptide which was discovered in 1999 (Kojima et al., 1999). Ghrelin is produced primarily in the stomach (Asakawa et al., 2005) and to a lesser extent in the bowel (Date et al., 2000), pancreas (Date et al., 2002), kidneys (Mori et al., 2000), testes (Tena-Sempere et al., 2002), lungs (Volante et al., 2002) and hypothalamus (van der Lely et al., 2004). Ghrelin exists in two forms, with approximately 80-90% being unacylated and 10-20% acylated. Ghrelin needs to be acylated in order to cross the blood-brain barrier and bind to growth hormone-secretagogue receptors (Asakawa et al., 2005). Thus, unacylated ghrelin is not thought to be implicated in the regulation of food intake, but does posses other actions such as adipogenesis.

Central ghrelin administration induces feeding in rats (Nakazato et al., 2001, Wren et al., 2001b) and intravenous infusion of ghrelin increases hunger and food intake in humans (Wren et al., 2001a; Figure 2.4). Furthermore, circulating concentrations of total and acylated ghrelin rise before food intake and decrease after a meal has been consumed (Cummings et al., 2001, 2002, Hosoda et al., 2004,

Lucidi et al., 2004, Blom et al., 2006). Thus, the available evidence strongly indicates that acylated ghrelin is a potent orexigenic peptide.

Figure 2.4. Effect of intravenous infusion of ghrelin on energy intake from a buffet. *** indicates significant ($P<0.05$) difference between conditions (Wren et al., 2001a).

In 1994, the product of the *ob* gene was identified and named leptin (Zhang et al., 1994). Leptin is a proteotypic peptide which is secreted primarily in adipocytes (Kirchgessner et al., 1997, Kraemer et al., 2002) and to lesser extent in gastric epithelium (Bado et al., 1998) and placenta (Masuzaki et al., 1997) and released into the circulatory system in proportion to adipose tissue mass (Frederich et al., 1995, Maffei et al., 1995). Leptin receptors are expressed in many parts of the brain, with the highest levels being present in mediobasal hypothalamus, a region which

regulates energy balance (Elmquist et al., 1998, Leshan et al., 2006). Thus, it is not surprising that leptin has been implicated in the control of energy intake.

Mice with a mutation of the *ob* gene secrete less leptin and mice with a mutation of the *db* gene express fewer leptin receptors when compared to normal mice (Coleman, 1978, Friedman and Leibel, 1992, Friedman and Halaas, 1998). Both mutations lead to excess adiposity. Furthermore, chronic administration of leptin to normal mice significantly reduces their food intake and adiposity (Halaas et al., 1995). Moreover, humans who do not have a correctly functioning *ob* gene are morbidly obese (Montague et al., 1997), fortunately body mass loss occurs in these individuals after chronic leptin administration (Farooqi et al., 1999, Farooqi et al., 2002, Gibson et al., 2004). Researchers have also shown with obese humans that combining a low-calorie diet with leptin treatment is more effective for body mass loss than a low-calorie diet alone (Heymsfield et al., 1999, Hukshorn et al., 2003). Thus, the available evidence robustly suggests that leptin is a potent anorectic hormone.

As a result of the above evidence regarding energy intake regulation, scientists have explored if problems (e.g., sleep disturbances and circadian misalignment) associated with shift-work are related to ghrelin and leptin. These studies are discussed below.

2.4.2.1.2 Desynchronisation, ghrelin and leptin

Research using both sleep diaries and laboratory equipment (e.g., using electroencephalography) has shown that shift-workers, when compared to day-workers, sleep significantly less (1-4 h) and are more likely to experience prolonged sleep latency (Tepas and Carvalhais, 1990, Ohayon et al., 2002, Akerstedt, 2003;

Figure 2.5). Moreover, in ~10% of night-workers, sleep-wake problems are severe enough to be clinically diagnosed as shift-work sleep disorder which is defined as excessive sleepiness during night-work and insomnia when attempting to sleep in the daytime (Drake et al., 2004). Thus, the available evidence strongly indicates that shift-work is a risk factor for sleep related problems.

Figure 2.5. Duration of the main sleep episode by groups and shifts. *P<0.05 with fixed daytime work schedules (Ohayon et al., 2002).

Cross-sectional studies have reported that total sleep duration is inversely related to circulating ghrelin concentration but positively associated with serum/plasma leptin levels (Taheri et al., 2004, Chaput et al., 2007). Moreover, an experimental study demonstrated that chronic sleep deprivation (4 h of sleep for six consecutive nights), when compared to 'normal' sleep (8 h of sleep for six consecutive nights), significantly reduces circulating leptin level (Spiegel et al., 2004a). Furthermore, another experimental study illustrated that acute sleep

deprivation (4 h of sleep for two consecutive nights), when compared to 10 h of sleep for two consecutive nights, significantly reduces circulating leptin level, increases plasma ghrelin concentration, elevates hunger and increases cravings for sweets (Spiegel et al., 2004b). Moreover, some researchers, using either a cross-sectional (Vioque et al., 2000, Shigeta et al., 2001, Heslop et al., 2002, Kripke et al., 2002, Amagai et al., 2004, Cournot et al., 2004, Taheri et al., 2004, Tamakoshi and Ohno, 2004, Singh et al., 2005, Björkelund et al., 2005) or prospective (Hasler et al., 2004, Gangwisch et al., 2005, Patel et al., 2006, Chaput et al., 2008) study design have demonstrated an association (normally curvilinear; i.e., a U-shaped relationship) between chronic sleep restriction and body mass index in adults, whereas others have observed no relationship (Gortmaker et al., 1990, Amagai et al., 2004, Lauderdale et al., 2006). The findings of some cross-sectional (e.g., Gortmaker et al., 1990, Kripke et al., 2002) and prospective studies (e.g., Hasler et al., 2004, Chaput et al., 2008) should be viewed with caution because sleep data were normally obtained via self-report questionnaires rather than via objective methods (e.g.,actigraphy). Scheer et al. (2009) recently demonstrated, using a forced desynchronisation protocol, that misalignment of the sleep-wake cycle with the body clock cycle (e.g., sleep between 12:00-20:00 h) reduces circulating leptin level by 17% compared to normal alignment (e.g., sleep between 00:00-08:00 h).

2.6. Shift-work and psychosocial disorders

Some researchers, using a cross-sectional study design, have reported that shift-work is positively associated with poor general mental health (Poole et al., 1992, Martens et al., 1999, Bardasi, 2000, Costa et al., 2004, Suzuki et al., 2004), stress (Gordon et al., 1986), irritation/strain (Frese and Semmer, 1986) anxiety and

depressive symptoms (Scott et al., 1997) whereas others have not reported an association between flexible work schedules, general mental health (Parkes, 1999) and depressive symptoms (Skipper et al., 1990). Clearly the abovementioned studies provide no indication of causality, but it is unlikely that individuals that have psychological problems actively seek out employment involving flexible work schedules. Nevertheless, epidemiologists using a prospective study design have reported that shift-work is a risk factor for poor general mental health, anxiety and depressive symptoms (Bildt and Michelsen, 2002, Bara and Arber, 2009). It is difficult to explain exactly why shift-workers seem more likely to suffer from psychological problems than day-workers, but it may due to several reasons: (i) shift-work is a risk factor for sleep problems and this is associated with psychological disorders such as depression (Walsh, 2004); (ii) shift-workers have difficulty fitting their lives around friends and family that lead a diurnal existence, thus shift-workers miss social events such as sporting events and parties which may lead to poor mental health (Albertsen et al., 2007); (iii) female shift-workers often have to prepare family meals at times to coincide with the diurnal existence of family members when the worker would rather rest and this may lead poor psychological well-being (Beermann and Nachreiner, 1995, Yildirim and Aycan, 2008). Overall, it appears that shift-work is a risk factor for psychological problems.

A popular belief is that obesity and mental health are related (Talen and Mann, 2009) and therefore one may think that shift-work is risk factor for excess obesity because working flexible schedules is associated with a range of psychological problems. Cross-sectional studies have generally observed a weak relationship between depression and obesity (Friedman and Brownell, 1995, Faith et al., 2002, Talen and Mann, 2009), although a significant relationship has been

observed in individuals with severe obesity (BMI>40 $kg \cdot m^2$) (Dixon et al., 2003, Onyike et al., 2003, Jia and Lubetkin, 2005). Nevertheless, such studies cannot determine if depression causes obesity or vice versa. Researchers using a prospective study design have reported that depression does cause body mass gain (Pine et al., 1997, 2001, Goodman and Whitaker, 2002, Roberts et al., 2003, Forman-Hoffman et al., 2007), whereas others have reported either no relationship (Stice et al., 2005) or that depression triggers body mass loss (DiPietro et al., 1992). Moreover, a recent meta-analysis concluded that depression does cause body mass gain (Blaine, 2008). However, it should be noted that the majority of studies in this area have followed adolescents into adulthood, thus it is still unclear if adult onset depression causes body mass gain. Moreover, cross-sectional (Nishitani and Sakakibara, 2006, Kouvonen et al., 2005) and prospective (Toyoshima et al., 2009) studies demonstrate a positive association between stress (a problem associated with shift-work) and excess adiposity. Furthermore, a recent prospective study demonstrated that general mental ill health increases the development of excess adiposity (Kivimaki et al., 2009). Psychological disorders are believed to cause excess adiposity by increasing the frequency of 'emotional eating', consuming energy dense foods and decreasing physical activity levels (Oliver and Wardle, 1999, Dallman et al., 2005, Wise et al., 2006). Overall, the available evidence indicates that some shift-work related psychological problems cause the development of excess adiposity.

2.4.2.1.4 Shift-work and energy intake

Due to shift-work related problems discussed above (e.g., sleep debt), one may expect the shift-worker to consume more energy than the day-worker. However,

some researchers have reported no difference between shift- and day-workers in regard to total 24 h energy intake (Lennernas et al., 1994, Reeves et al., 2004), whereas others have reported that shift-workers consume less energy over a given 24 h period than day-workers (Takagi, 1972, Sudo and Ohtsuka, 2001). Furthermore, Lennernas et al. (1994) found no difference between shift- and day-workers in regard to energy intake from macronutrients, whereas Sudo and Ohtsuka (2001) reported that shift-workers ingest less protein, carbohydrate and fat than day-workers. The findings of the abovementioned studies need to be viewed with caution because the methods used to assess habitual dietary habits (e.g., a 24 h recall diary) are associated with significant measurement error (Trabulsi and Schoeller, 2001). Overall, current data regarding total energy and macronutrient intake are equivocal. Thus it is unclear if the positive association between shift-work and excess adiposity is because shift-worker's total energy is greater than day-worker's.

2.4.2.1.5 Shift-work and physical activity habits

Sufficient energy expenditure via physical activity can prevent the development of excess adiposity. Thus, one may think that shift-workers undertake less physical activity than day-workers and that this explains why working flexible schedules is a risk factor for excess adiposity. Researchers using a cross-sectional study design have reported no difference between shift- and day-workers in regard to physical activity habits (De Backer, 1987, Costa, 1990, Kivimaki et al., 2001), whereas other scientists have reported that female shift-workers undertake more exercise than female day-workers (Lasfargues et al., 1996). Prospective studies have shown no difference between shift- and night-workers in regard to physical activity habits (Theorell and Akerstedt, 1976), whereas others have shown that shift-workers

undertake less exercise than day-workers (Nakamura et al., 1997). Kawachi et al. (1995) reported that shift-work experience was positively associated with physical activity level. The findings the abovementioned studies need to be viewed with caution because habitual physical activity was measured via primitive questionnaires. For example, the physical activity data of Nakamura et al. (1997) is based upon a single question regarding frequency of exercise: never, 1-3 per month, 1-2 times per week, >2 times per week or every day. Such a question provides no information regarding duration of physical activity which is important when attempting to determine if physical activity differs between two populations. Future research should objectively measure habitual physical activity via activity monitors. Overall, the available evidence regarding whether shift-work affects physical activity habits is inconclusive and therefore it is unclear if the effect of flexible work schedules on adiposity is mediated through less energy, compared to day-workers, being expended via physical activity.

2.4.2.1.6 Temporal position of food intake and adiposity

Shift-workers are more likely to consume food late in the evening or at night than day workers. This effect of shift-work on temporal positioning of food intake may contribute to the positive association between flexible work schedules and adiposity. For example, using a cross-sectional study design, researchers have shown that individuals who consume food at night are more likely to have excess adiposity than people who do not, independent of other risk factors for excess adiposity such as physical activity level and total energy intake (Ma et al., 2003, Berg et al., 2009). Moreover, epidemiologists, using a prospective study design, demonstrated an inverse relationship between energy intake at breakfast and body mass, independent

of physical activity habits and total energy intake (Purslow et al., 2008). These findings may seem perplexing considering energy intake and expenditure via physical activity has been accounted for. However, in individuals living a 'normal' diurnal existence, diet-induced thermogenesis is significantly lower in the morning, compared to the afternoon and especially night time (Romon et al., 1993; Figure 2.7). The exact reason for the abovementioned finding is unknown but may to due to the following. The facultative component of diet-induced thermogenesis is modulated by futile substrate cycling, protein turnover and sodium pumping, all of which are influenced by sympathetic outflow because aforementioned facultative processes are suppressed by β–andrengic receptor blockade (Acheson et al., 1984). Epinephrine-induced thermogenesis is suppressed by insulin concentrations (Muller et al., 1992), which are higher at rest and postprandially at night compared to the day (Van Cauter et al., 1992, Al-Naimi et al., 2004) and thus this may explain the findings of Romon et al. (1993).

Furthermore, Arble et al. (2009) fed mice for 6 weeks either in the 12 h light phase or the 12 h dark phase. Mice fed during the light phase gained significantly more body mass and fat than mice fed during the dark phase. The light fed mice consumed more energy and undertook less locomotor activity than the dark fed mice, although these group differences did not reach statistical significance. Thus, it is possible that the additive effects of more energy being consumed and less locomotor activity being undertaken in the light fed mice, than the dark fed mice, caused the group differences in regard to body mass and fat. Moreover, it is possible that diet-induced thermogenesis was lower in the mice fed at the 'wrong time' (e.g., light fed mice) than 'correct time' (e.g., dark fed mice) as shown by Romon et al. (1993) in humans.

Overall, these findings discussed in this section indicate that circadian timing of food intake is an important factor in regard to energy balance.

Figure 2.7. Patterns of energy expenditure after ingestion of a meal at different times of day for a 60 min period of measurement (Romon et al., 1993). Data are expressed as mean±SEM.

2.4.2.1.7 Summary of possible mechanisms underlying the adverse association between shift-work and adiposity

The available evidence indicates that sleep curtailment and temporal repositioning of the sleep-wake cycle relative to the body clock causes unfavourable alterations in ghrelin and leptin which may lead to excessive food intake. Moreover, psychological

problems associated with shift-work may also lead to excessive intake of calories. However, it is still unclear if shift-workers actually consume more energy than day-workers. Currently, it is unclear if shift-workers expend more, less or the same amount of energy via physical activity than day-workers. Shift-workers are more likely to consume food at night than day workers and this may lead to lower diet-induced thermogenesis. Thus, the unfavourable effects of shift-work discussed in regard to sleep, psychology and temporal positioning of food intake may explain why individuals that undertake flexible work schedules are at greater risk of having excess adiposity than day-workers. Clearly, interventions which alleviate one or more of the abovementioned adverse consequences of shift-work should be explored in order to help this population. One such intervention may be physical activity.

2.4.3 Shift-work and gastrointestinal symptoms

Many factors may explain the association between shift-work and gastrointestinal problems. Researchers using a cross-sectional study design have reported an inverse association between sleep and gastrointestinal problems (Jarrett et al., 2000, Fass et al., 2000, Vege et al., 2004, Lu et al., 2006, Nojkov et al., 2008, Cremonini et al., 2009). Moreover, a prospective study indicates that sleep disturbance causes gastrointestinal problems (Goldsmith and Levin, 1993). Most of the the abovementioned studies cannot determine causality, but there are some adverse effects of sleep disturbance which may explain why poor sleep could cause gastrointestinal problems. For example, (i) total and modest sleep deprivation leads to hyperalgesia in response to a painful stimulation applied to the hand (Onen et al., 2001, Roehrs et al., 2006); (ii) sleep deprivation has been reported to increase

symptom intensity rating in response to oesophageal acid perfusion (Schey et al., 2007) and (iii) acute and chronic sleep disruption exacerbates colonic inflammation in mice (Tang et al., 2009). Shift-work appears to cause sleep problems (see above) and this may explain why working flexible schedules is associated with gastrointestinal problems.

Many factors involved in the gastrointestinal system are controlled by body clock (Hoogerwerf, 2009). For example, gastrointestinal motility is greater in the day than at night (Kumar et al., 1986, Goo et al., 1987, Auwerda et al., 2001, Hoogerwerf et al., 2009) and digestive enzyme expression varies with time of day (Hoogerwerf, 2006). To date, no researcher has objectively investigated the effect of circadian misalignment (as occurs with shift-work) on factors involved in the gastrointestinal system. Thus, it is unclear if shift-work causes gastrointestinal problems by disrupting factors that regulate the digestive system.

2.4.3.1 Summary of possible mechanisms underlying the adverse association between shift-work and gastrointestinal health

Available evidence indicates that disturbed sleep may be the cause of gastrointestinal problems in shift-workers. However, well controlled experiments that investigate the effect of circadian misalignment upon factors related to gastrointestinal health (e.g., colonic motility) are desperately needed.

2.5 PHYSICAL ACTIVITY AND ADIPOSITY

Many studies have explored the relationship between physical activity and body mass. These studies are reviewed below.

2.5.1 Physical activity and primary prevention of body mass gain in day-workers

Prospective studies that have analysed the relationship between physical activity and body mass can be categorised depending on when physical activity data were obtained; that is, whether baseline, follow-up or change in physical activity was compared against change in body mass (Fogelholm et al., 2006). Findings from longitudinal studies using baseline physical activity data are conflicting. For example, researchers have reported that over time, depending on gender, physically active rather than inactive individuals gain less (Klesges et al., 1992, Owens et al., 1992), more (Klesges et al., 1992, Bild et al., 1996, Haapanen et al., 1997) or similar (Williamson et al., 1993, Haapanen et al., 1997, Parker et al., 1997) amounts of body mass. Epidemiologists using physical activity data at follow-up generally report an inverse relationship between physical activity and weight gain (Rissanen et al., 1991, Williamson et al., 1993, Barefoot et al., 1998), although one research group found no association (Heitmann et al., 1997). Researchers analysing a change (from baseline to follow-up) in physical activity have reported an inverse relationship between physical activity and body mass gain (Owens et al., 1992, Williamson et al., 1993, Taylor et al., 1994, Haapanen et al., 1997), although some scientists have observed no association (Bild et al., 1996, French et al., 1999).

Some epidemiologists who have employed a randomised controlled trial have shown physical activity can attenuate body mass gain in individuals (Donnelly et al., 2003a, McTiernan et al., 2007), whereas others have observed no effect of physical activity on people's body mass (Schmitz et al., 2003). These conflicting findings are probably due to differences in physical activity intervention; Donnelly et al. (2003a) and McTiernan et al. (2007) used an intervention consisting of aerobic exercise,

whereas Schmitz et al. (2003) employed a resistance training intervention which is normally associated with muscle mass gain rather than fat loss.

2.5.2 Physical activity for body mass reduction in day-workers

Numerous randomized controlled trials have been undertaken to determine if physical activity induces body mass loss in individuals. Some researchers have reported that individuals who undertake physical activity significantly reduce their body mass compared to sedentary people (Wood et al., 1983, 1988, Ronnemaa et al., 1988, Hammer et al., 1989, Hellenius et al., 1993, Anderssen et al., 1995, Donnelly et al., 2003a, Campbell et al., 2007), whereas others have observed no effect of physical activity on body mass (Verity and Ismail, 1989, King et al., 1991, Katzel et al., 1995, Dengel et al., 1998, Stefanick et al., 1998). A meta-analysis and review (Wing, 1999), although dated, concluded that physical activity results in a modest reduction in body mass, ranging from 1 to 3 kg. However, the aforementioned reports did not include the study conducted by Donnelly et al. (2003a), in this investigation all physical activity sessions were supervised over 16 months to ensure participants adhered to the study intervention. Men in the intervention group lost 4.8 kg more of body mass than individuals in the control group.

2.5.3 Physical activity for maintenance of reduced body mass in day-workers

Scientists using a prospective study design report that individuals who have lost body mass via some strategy (e.g., surgery) regain less body mass over time if they are physically active rather than sedentary (Sikand et al., 1988, Pavlou et al., 1989, Holden et al., 1992, Depue et al., 1995). These findings have also been confirmed by

randomised controlled trials (Perri et al., 1988, Fogelholm et al., 2000). Moreover, intervention studies indicate that the more physical activity an individual undertakes, the less body mass they regain (Andersen et al., 1999, Jakicic et al., 1999, Jakicic et al., 2003).

2.5.4 Physical activity and adiposity in shift-workers

Harma et al. (1988) are the only research group that has explored the relationship between physical activity and adiposity specifically in shift-workers. Using a randomised controlled trial, Harma et al. (1988) found that a 4-month supervised physical activity program had no effect on body mass and skinfold data. Moreover, Elliot et al. (2007), using a randomised controlled trial, reported that motivational interviewing, which focussed upon increasing fire-fighter's physical activity level and improving dietary habits, significantly reduced body mass gain over time.

The abovementioned investigations provide useful data applicable to shift-workers, however only one of these studies (Elliot et al., 2007) utilised an individualised program. An individualised lifestyle program is possibly more suitable than an inflexible or supervised one for shift-workers because their work-rest schedules are often altering. The study by Elliot et al. (2007) involved US fire-fighters. It is possible that such workers are more likely to be ready to change their physical activity habits due to their job requiring a degree of physical fitness. Furthermore, in general, fire-fighters are allowed to sleep during the night-shift if not called out to an emergency. Consequently, the adverse effects of nocturnal wakefulness might not apply as readily to fire-fighters as to call centre workers or nurses for example.

2.5.5 Summary of relationship between physical activity and adiposity

Available evidence indicates that physically active individuals gain less body mass over time. A recent review suggested that moderately vigorous physical activity of 150 to 250 min·wk^{-1} with an energy equivalent of 1200 to 2000 kcal·wk^{-1} is sufficient to prevent body mass gain in most adults (Donnelly et al., 2009).

Past data also suggests that physical activity is an effective tool for attenuating body mass regain in individuals. The available data indicate a dose-response relationship between physical activity and body mass regain.

Moreover, research suggests that physical activity can cause body mass loss in overweight and obese individuals. Current data indicate that a dose-response relationship exists between physical activity and body mass loss: physical activity <150 min·wk^{-1} results in no body mass loss, physical activity >150 min·wk^{-1} causes a loss of body mass ranging between 2 to 3 kg and physical activity between 225 and 420 min·wk^{-1} causes a reduction in body mass between 5 to 7.5 kg (Donnelly et al., 2009).

Despite the wealth of research regarding physical activity and adiposity, the majority of past research has involved day-workers. Thus, it is still unclear if physical activity can help control UK shift-worker's adiposity.

2.6 MECHANISMS UNDERLYING FAVOURABLE EFFECTS OF PHYSICAL ACTIVITY ON ADIPOSITY

Physical activity causes an increase in energy expenditure and may suppress hunger which could lead to a reduction in calorie intake. These two responses to exercise are discussed below.

2.6.1 Physical activity and energy expenditure

In lean and obese individuals, an acute bout of exercise increases lipolysis (hydrolysis of triacylglycerol) in adipose tissue and raises lipid oxidation in skeletal muscle (Horowitz and Klein, 2000, Stich et al., 2000, van Hall et al., 2002, Mittendorfer et al., 2004, Richterova et al., 2004). The exercise-mediated increase in adipose lipolysis causes the loss of adipose tissue (Ross and Janssen, 2001, Donnelly et al., 2003b, Gan et al., 2003, Irwin et al., 2003, Slentz et al., 2004) and the amount lost is positively related to the energy cost of exercise (Ross and Janssen, 2001, Irwin et al., 2003, Slentz et al., 2004). Acute exercise stimulates adipose tissue lipolysis to a greater extent in the abdomen than femoral or gluteal adipose tissue (Arner et al., 1990, Horowitz et al., 2000). This may be due to lipolytic sensitivity to catecholamines (hormones which promote lipolysis) being greater in intra-abdominal than limb tissue (Fogelholm et al., 2006). However, scientists have shown that the relative training-induced loss of adipose tissue from abdomen and limbs is similar (Gan et al., 2003, Slentz et al., 2004). Moreover, researchers have reported that relative training-induced loss of visceral compared to subcutaneous adipose tissue is greater (Ross et al., 2000, 2004, Gutin et al., 2002) or less (Donnelly et al., 2003a) or the same (Irwin et al., 2003).

Overall, the available evidence indicates that physical activity, relative to the energy cost of the activity, promotes lipolysis in adipose tissue which causes a reduction in body fat. Relative training-induced adipose tissue loss is similar in the abdomen and limbs. However, the relative physical activity mediated loss of visceral compared to subcutaneous adipose tissue is greater. Thus, the above processes explain how physical activity primarily affects body mass.

2.6.2 Physical activity and energy intake

Many researchers have investigated the effect of an acute bout of physical activity upon circulating concentrations of ghrelin and leptin because these two factors regulate hunger. These studies are discussed below.

2.6.2.1 Physical activity and ghrelin

Some scientists have reported that during and post-exercise, circulating total ghrelin concentration is increased (Christ et al., 2006, Erdmann et al., 2007, Jurimae et al., 2007b, Sartorio et al., 2008), decreased (Kraemer et al., 2004b, Ghanbari-Niaki, 2006, Toshinai et al., 2007, Vestergaard et al., 2007, Malkova et al., 2008, Ballard et al., 2009) or unaffected (Dall et al., 2002, Kallio et al., 2001, Kraemer et al., 2004a, Schmidt et al., 2004, Takano et al., 2005, Pomerants et al., 2006, Burns et al., 2007, Jurimae et al., 2007a, Martins et al., 2007). It is difficult to determine why the abovementioned studies provide conflicting data, but it may be due to between study differences in exercise intensity or participants being fasted or fed. Nevertheless, Broom et al. (2007) noted that the measurement of total ghrelin may mask important changes in acylated ghrelin, the form of the hormone which increases hunger. Consequently, Broom et al. (2007, 2009) and Marzullo et al. (2008) investigated the effect of physical activity on plasma acylated ghrelin concentration. They reported that plasma acylated ghrelin is suppressed during and post-exercise. In contrast, Mackelvie et al. (2007) reported that five consecutive days of aerobic exercise (1 h/day) increases fasting and postprandial plasma acylated ghrelin concentrations. In the study by Mackelvie et al. (2007) the time elapsed between the last exercise session and the measurement of acylated ghrelin was at least 10 h which contrasts with the studies by Broom et al. (2007, 2009) and Marzullo et al. (2008) in which

acylated ghrelin was measured during and immediately post-exercise. This may explain the contrasting findings and also indicates that the effect of physical activity on acylated ghrelin is transient.

Many mechanisms may explain why an acute bout of physical activity suppresses circulating acylated ghrelin level during and post-exercise. Insulin is known to suppress circulating levels of ghrelin (Flanagan et al., 2003) and insulin sensitivity (Hansen et al., 1998) is increased by a bout of physical activity of sufficient intensity and duration. However, Broom et al. (2007, 2009) reported no effect of exercise on circulating insulin concentrations. One may believe this indicates that the effect of exercise upon acylated ghrelin is not mediated through an increase in insulin sensitivity. However, it is possible that insulin secretion and sensitivity were increased concurrently and therefore the exercise-mediated suppression of acylated ghrelin may have been caused by alterations relating to insulin.

Circulating glucose and non-esterified fatty acids (NEFA) are also known to suppress circulating ghrelin level (Shiiya et al., 2002, Gormsen et al., 2006) and these metabolites normally increase during and after exercise (van Hall et al., 2002, Broom et al., 2007, 2009, Enevoldsen et al., 2007). Moreover, acromegalic patients have lower levels of circulating total ghrelin than healthy individuals (Cappiello et al., 2002, Freda et al., 2003), indicating that growth hormone, which increases in response to physical activity (Felsing et al., 1992), suppresses the aforementioned orexigenic hormone. Thus, it is possible that the effect of exercise upon plasma acylated ghrelin level is mediated through an increase in circulating glucose, NEFA and/or growth hormone levels.

2.6.2.2 Physical activity and leptin

Studies regarding the effect of physical activity on circulating leptin concentration can be divided into four categories: (i) short-term (<60 min) exercise; (ii) long-term (≥60 min) exercise; (iii) short-term (<12 weeks) training and (iv) long-term (≥12 weeks) training. These categories are discussed below.

Researchers have reported that circulating leptin level is increased (Kraemer et al., 2001), decreased (Elias et al., 2000, Legakis et al., 2004, Jurimae and Jurimae, 2005) or unaffected (Kraemer et al., 1999a, Weltman et al., 2000, Fisher et al., 2001, Zafeiridis et al., 2003, Sari et al., 2007) after short-term physical activity. Moreover, scientists have noted that during and after long-term exercise circulating leptin level is reduced (Landt et al., 1997, Tuominen et al., 1997, Koistinen et al., 1998, Leal-Cerro et al., 1998, Duclos et al., 1999, Essig et al., 2000, Olive and Miller, 2001, Zaccaria et al., 2002, Desgorces et al., 2004) or unchanged (Hickey et al., 1996, Racette et al., 1997, Torjman et al., 1999, Olive and Miller, 2001). Short-term training has been reported to decrease (Ishii et al., 2001) or have no effect (Dirlewanger et al., 1999, Kraemer et al., 1999b, 2001, Houmard et al., 2000) on fasting circulating leptin level. Researchers have reported that long-term training decreases (Kohrt et al., 1996, Hickey et al., 1997, Perusse et al., 1997, Reseland et al., 2001) or has no effect (Noland et al., 2001) on circulating fasting leptin level. It is worth noting that some of the abovementioned studies have limitations; for example, some researchers (e.g., Elias et al., 2000) did not use a control trial and thus their leptin data may be affected by diurnal variation *per se* rather physical activity, some studies (e.g., Kraemer et al., 2001) did not account for heamoconcentration which can 'artificially' raise the concentration of a hormone in a plasma/serum sample.

Recent work involving rats suggests that acute physical activity can also increase hypothalamic sensitivity to leptin (Flores et al., 2006).

Leptin is secreted primarily by adipose tissue (Kraemer et al., 2002), thus reducing an individual's body fat should reduce their circulating level of leptin. As discussed above, a negative energy balance induced by physical activity causes the loss of adipose tissue (Gan et al., 2003, Irwin et al., 2003) and this may explain why long-term exercise and training reduces fasting circulating leptin level. Indeed, researchers who have accounted for the energy cost of exercise directly (i.e., by increasing energy intake) or by using body fat loss data as a covariate in a statistical analysis report no effect of physical activity on circulating leptin concentration (van Aggel-Leijssen et al., 1999, Hilton and Loucks, 2000). Insulin is known to increase leptin secretion from adipocytes (Malmstrom et al., 1996, Utriainen et al., 1996). As stated before, exercise training can improve insulin sensitivity which may reduce circulating levels of insulin (Houmard et al., 2004) and therefore this could also explain why long-term exercise training reduces circulating leptin levels.

2.6.2.3 Physical activity, hunger and food intake

Although there is evidence indicating that exercise does affect factors that control hunger (see above), it is worth reviewing studies that investigated the effect of physical activity on hunger and food intake. Researchers have reported that an acute bout of exercise suppresses (Thompson et al., 1988, King et al., 1994, King and Blundell, 1995, Westerterp-Plantenga et al., 1997, Broom et al., 2007, 2009) or increases (Lluch et al., 1998, Maraki et al., 2005) hunger. These discrepancies may be due to between study differences in regard to exercise intensity, fitness status of participants and whether subjects were in a fasted state or not.

Researchers, using an experimental design, have reported that an acute bout of exercise increases (Verger et al., 1992, 1994, Lavin et al., 1998), decreases (Westerterp-Plantenga et al., 1997) or has no effect on (Jankowski and Foss, 1972, Thompson et al., 1988, Horton et al., 1994, King et al., 1994, 1996, 1997, Tremblay et al., 1994, Almeras et al., 1995, King and Blundell, 1995, Imbeault et al., 1997, Gilsenan et al., 1998, Lluch et al., 1998) *ad labitum* food intake. Moreover, experimental data indicate that short-term exercise training (2 to 7 weeks) can increase (Woo and Pi-Sunyer, 1985), decrease (Dickson-Parnell and Zeichner, 1985, Martins et al., 2007) or have no effect on (Epstein et al., 1978, Woo et al., 1982a, 1982b, Keim et al., 1990) *ad labitum* energy consumption. Data from intervention studies indicate that long-term exercise training (2 to 18 months) may increase (Janssen et al., 1989), decrease (Johnson et al., 1972, Watt et al., 1976, Leon et al., 1979, Tagliaferro et al., 1986, Wood et al., 1988, Broeder et al., 1992) or have no effect (Dempsey, 1964, Holloszy et al., 1964, Wood et al., 1983, Andersson et al., 1991, van Etten et al., 1997) on freely chosen food intake. Evidence exists which indicates that gender and body mass modulate the effect of exercise on energy intake: females sometimes increase their post-exercise energy intake which either reduces or eradicates the negative energy balance induced by physical activity (Westerterp et al., 1992, Pomerleau et al., 2004); lean individuals sometimes (Durrant et al., 1982), but generally do not, increase their post-exercise energy intake whereas obese individuals have no compensatory response. Inter-study differences regarding exercise intensity, nutritional state of individuals, method used to measure food intake and time lapse between physical activity and eating may explain the conflicting findings between the above investigations (Martins et al., 2008). It is worth noting that the abovementioned studies have limitations, for

example energy intake was measured via a food diary in many studies and this method is associated with a certain degree of error.

2.6.3 Summary of mechanisms underlying favourable effects of physical activity on adiposity

An acute bout of exercise suppresses circulating acylated ghrelin concentration. In regard to circulating leptin level, the available evidence is equivocal in regard to the effect of short-term exercise, however short-term training appears to have no effect but long-term exercise and training reduces fasting levels of the hormone. However, the exercise-mediated suppression of leptin may be countered by hypothalamic sensitivity to leptin increasing in response to physical activity. Evidence indicates that an acute bout of exercise suppresses hunger which may be due, in part, to the exercise-mediated suppression of acylated ghrelin and/or an increase in hypothalamic sensitivity to leptin. The effect of exercise on hunger may explain why acute, short- and long-term exercise does not increase food intake and therefore can induce a negative energy balance and thus prevent body mass gain and induce body mass loss. Notably, all previous work regarding physical activity, ghrelin, leptin and hunger has been undertaken during the day-time and thus it unclear how the aforementioned factors respond during nocturnal wakefulness (e.g., a simulated night-shift) when evening exercise has been undertaken.

2.7 PHYSICAL ACTIVITY AND GASTROINTESTINAL HEALTH

The body of literature regarding physical activity and gastrointestinal is somewhat small. Nevertheless, an acute bout of physical activity has been reported to cause heartburn in some individuals (Clark et al., 1989, Peters et al., 1999b, 2001). Moreover, some researchers using a cross-sectional study design have reported an

inverse relationship between physical activity and constipation (Donald et al., 1985, Sandler et al., 1990, Dukas et al., 2003), whereas others have observed no relationship (Klauser et al., 1992, Tuteja et al., 2005). Data from a non-randomised-controlled study indicated no effect of physical activity training on frequency of constipation (Meshkinpour et al., 1998), however the findings of a fully randomised-controlled study indicated that physical activity training reduces frequency of constipation (Daley et al., 2008). Nausea, bloating, abdominal pain, flatulence and diarrhoea are reported during and/or after prolonged physical activity (e.g., a marathon) in some individuals (Brouns & Beckers, 1993; Peters et al., 1999a).

Gastro-oesophageal acid reflux is induced by an acute bout of exercise (Clarke, 1989) and this may explain why physical activity causes heartburn in some people. However, Peters et al. (1999b) reduced running induced gastro-oesophageal acid reflux by administering omeprazole, however heartburn was still induced by the bout of exercise. Thus, the study by Peters et al. (1999b) suggests that the importance of gastro-oesophageal acid reflex in exercise-mediated heartburn is probably minimal. Simren (2002) postulated that factors such as non-acid gastro-oesophageal reflux, musculoskeletal problems and mechanical distension of the oesophagus from aerophobia may also cause heartburn (Simrén, 2002). Physical activity decreases mouth-to-anus transit time and this may explain why researchers have reported an inverse relationship between exercise and constipation. The exercise-mediated decrease in mouth-anus transit time may be caused by alterations in gut blood flow, hormones (e.g., motilin), neurogenics or mechanical bouncing of gastrointestinal contents (Peters et al., 2001).

2.7.1 Physical activity and gastrointestinal health in shift-workers

Harma et al. (1988) are the only scientists that have investigated the association between physical activity and frequency of gastrointestinal symptoms in shift-workers. Harma et al. (1988) reported a significantly greater post-study reduction in gastrointestinal symptoms in shift-workers randomised to the control group (-0.7%) rather than the physical activity intervention (0%). This finding is illogical and should be viewed with caution.

2.7.2 Summary of physical activity and gastrointestinal health

The available evidence indicates that an acute bout of exercise causes heartburn, nausea, bloating, abdominal pain, flatulence and diarrhoea in some individuals. However, exercise training does appear to reduce the frequency of constipation. It should be noted more studies are required to determine conclusively the effect of exercise training on gastrointestinal problems such as diarrhoea, bloating and abdominal pain. Only one research group has explored the relationship between physical activity and gastrointestinal symptoms in shift-workers and they provided odd findings. Thus, another exploration of the association between physical activity and digestive problems in shift-workers is warranted.

2.8 SYNOPSIS

Shift-work is prevalent in Western society and is a risk factor for adiposity and gastrointestinal symptoms. Research involving day-workers, indicates that physical activity can beneficently affect processes involved in energy storage (e.g., acylated ghrelin) which in turn leads to the prevention or reduction of adiposity. Moreover, physical activity may reduce the frequency of some gastrointestinal symptoms (e.g.,

constipation) in day-workers. However, there is lack of research regarding physical activity, adiposity and gastrointestinal health in shift-workers and therefore this provides the rationale for this thesis.

CHAPTER 3

RELATIONSHIPS BETWEEN LEISURE-TIME PHYSICAL ACTIVITY, BODY MASS INDEX AND DIGESTIVE SYMPTOMS IN SHIFT-WORKERS

3.1 INTRODUCTION

Shift-work is considered a risk factor for numerous health problems (Knutsson, 2003, Suwazono et al., 2008, Puttonen et al., 2009). For example, some researchers have reported that shift-workers have greater adiposity (Niedhammer et al., 1996, van Amelsvoort et al., 1999, Karlsson et al., 2001, Parkes, 2002, Di Lorenzo et al., 2003, Ishizaki et al., 2004, Morikawa et al., 2007, Suwazono et al., 2008) and more gastrointestinal problems (Angersbach et al., 1980, Costa et al., 1980, Smith and Colligan, 1982, Ottmann et al., 1989, Segawa et al., 1987, Zober et al., 1998) than day-workers, although others have not observed such findings relating to adiposity (Karlsson et al., 2003, Ha and Park, 2005b, Watari et al., 2006, Nabe-Nielsen et al., 2008, De Bacquer et al., 2009) and digestive symptoms (Costa et al., 1990, Poole et al., 1992, Jaffe et al., 1996). On balance, these findings are worrying considering that about 20% of Western society partakes in shift-work, there is a strong relationship between excess fat and morbidity or mortality, and that, digestive symptoms are associated with reduced quality of life (Damon et al., 2004). Clearly, an exploration of factors that may relate to adiposity and digestive symptoms in shift-workers is warranted.

Leisure-time physical activity (LTPA) is obviously associated with an increase in energy expenditure. Nevertheless, LTPA could also reduce energy intake via an exercise-related suppression of hunger (Broom et al., 2007, 2009). Data from prospective and randomised controlled trials generally indicate that LTPA can prevent body mass gain (Rissanen et al., 1991, Owens et al., 1992, Taylor et al., 1994, McTiernan et al., 2007) and reduce body mass regain in day-workers who have lost body mass via some strategy (Sikand et al., 1988, Pavlou et al., 1989, Holden et al., 1992, Depue et al., 1995, Fogelholm et al., 2000). Unfortunately, the

contribution of an exercise-related decrease in hunger has not been examined in these intervention studies.

It has also been postulated that physical activity improves digestive health by mediating a reduction in gastrointestinal blood flow, altering neuroimmuno-endocrine factors and/or increasing gastrointestinal motility (Peters et al., 2001). Despite these postulations, there are conflicting findings regarding physical activity and gastrointestinal symptoms. For example, an inverse association (Everhart et al., 1989, Sandler, 1990, Colwell et al., 1998, Brown et al., 2000, Lustyk et al., 2001, Dukas et al., 2003) and no relationship (Klauser et al., 1992, Tuteja et al., 2005) between physical activity and digestive symptoms have been reported in day workers. Data from a non-randomised-controlled study indicated no effect of physical activity on digestive symptoms in day workers (Meshkinpour et al., 1998), although the findings of another fully randomised-controlled study demonstrated that physical activity reduces frequency of constipation (Daley et al., 2008). It is possible that the day workers recruited for the above studies were very variable in the degree of gastro-intestinal disturbances at baseline, which may influence the consistency and generalizability of results.

No researcher has investigated the inter-relationships between LTPA, BMI and gastrointestinal problems in shift workers, which is surprising considering this population is more prone to these health problems than day workers. It is hypothesise that LTPA is inversely associated with BMI and frequency of digestive problems in shift workers.

3.2 METHODS

3.2.1 Study population

Approximately 500 questionnaires were distributed to several organisations over six months. One hundred and seventy one shift workers returned questionnaires in the pre-paid envelope that was supplied (response rate: 32%). The questionnaire was essentially the Standard Shift-Work Index (Barton et al., 1995) supplemented with a validated LTPA questionnaire (Lamb and Brodie, 1991). The respondents had been involved in a three-shift (morning, afternoon, nights), rotating system for >six months. The shift workers were fire fighters, midwives or police staff. This provided shift-workers who are asked to maintain some level of physically fitness for the purpose of their job (e.g., fire fighters) and other workers that are not (e.g., police call handlers or midwives). Characteristics of the shift workers are presented in Table 3.1. Approval for this study was granted by the local ethics committee.

Table 3.1. Characteristics of shift-workers studied.

Characteristics of shift-workers studied	
Number shift-workers	171
Number of males	107
Number of females	64
Mean (SD) age	39.0 (9.1) years
Mean (SD) experience of shift-work	148.1 (115.2) months
Mean (SD) height	1.7 (0.1) m
Mean (SD) body mass	80.6 (13.9) kg
Mean (SD) body mass index	26.6 (4.0) $kg \cdot m^2$
Marital status	
Married/living with partner	81.3%
Single	18.7%
Job type	
Fire fighters	40.1%
Police staff	44.4%
Midwives	14.6%

3.2.2 Leisure-time physical activity assessment

An adapted version of the Lamb and Brodie (1991) LTPA questionnaire was completed by each participant. This questionnaire was complemented with questions regarding time spent watching television, transportation, adherence to exercise regimes, availability/accessibility to exercise facilities and barriers to participating in LTPA. Shift workers recorded the duration and frequency of physical activities, from an extensive list, they had undertaken during their leisure-time in the previous 14 days. These data were used to determine, via metabolic tables, LTPA energy expenditure during the previous 14 days. Participants were then allocated to one of six groups: group 1 (0 to 1.1 MJ·14 days^{-1}), group 2 (1.2 to 6.3 MJ·14 days^{-1}) , group 3 LTPA (6.5 to 12.4 MJ·14 days^{-1}), group 4 (12.4 to 17.3 MJ·14 days^{-1}), group 5 (17.5 to 30.4 MJ·14 days^{-1}) and group 6 (31.1 to 173.4 MJ·14 days^{-1}).

3.2.3 Body mass index assessment

Participants self-reported their own height and body mass and these data were used to calculate BMI (kg·m^2). A systematic review found that self-reported height and body mass are slightly, yet systematically overestimated and underestimated, respectively (Gorber et al., 2007). Nevertheless, self-reported height and body mass data are satisfactory to be used in large cross-sectional studies (Dekkers et al., 2008).

3.2.4 Digestive health assessment

The digestive health questionnaire from the Standard Shift-Work Index (Barton et al., 1995) was completed by participants. This questionnaire comprised eight questions relating to the frequency (almost never, quite seldom, quite often, almost always) of

appetite disruption, avoidance of certain foods to prevent stomach upsets, nauseous, heartburn, digestion difficulties, bloated stomach or flatulence, constipation or diarrhoea and abdominal pain. Moreover, the data from these questions were summed to provide a total digestive health score.

3.2.5 Assessment of confounders

Physical, lifestyle, socio-demographic and job characteristics, such as age, gender, smoking status (smoker or non-smoker), alcohol consumption (units per week), marital status (married, co-inhabiting or single), job type (fire fighters, midwives or police staff) and shift work experience (months in shift work) were assessed via a questionnaire.

3.2.6 Statistical analysis

A preliminary exploration of the associations between BMI and digestive health symptoms with potential confounders (physical, lifestyle, socio-demographic and work characteristics) was undertaken using one-way ANOVA, Kruskall-Wallis test, Pearson's/Spearmen's correlation coefficients and ordinal regression. Differences in BMI and total digestive health score between LTPA groups were investigated with an analysis of covariance (ANCOVA) model, adjusting for the factors found to be related to these outcomes in the initial exploration (Table 3.2). Shift work experience was significantly associated with BMI, but was not included as a covariate due to its multicollinearity with age, which was entered as a covariate. Simple planned contrasts were performed with the least active participants as the comparator group. Associations between LTPA and frequency of individual digestive symptoms (e.g., heartburn) were explored with ordinal regression, adjusting for variables found to be

relevant to these outcome measures from the preliminary exploration. With respect to the digestive symptoms questions, the 'almost always' category was collapsed into the 'quite often' group in order to meet the adequate cell count assumption of ordinal regression. Reference categories used in ordinal regression analysis were 'almost never' and 'extremely high LTPA group'. All statistical procedures were computed by SPSS for Windows, version 15. Statistical significance was set at $P<0.05$.

Table 3.2. Statistical significance (exact *P*-values) from exploration of body mass index and digestive health symptoms associations with possible confounders.

Variable	BMI	DHQ1	DHQ2	DHQ3	DHQ4	DHQ5	DHQ6	DHQ7	DHQ8	TDHS
Age	<0.0005	0.117	**0.002**	0.054	0.276	0.218	0.629	0.616	0.041	0.141
Smoke	0.158	0.030	0.385	0.325	0.497	0.517	0.163	0.389	0.083	0.075
Alcohol	0.693	0.485	0.445	0.428	0.406	0.644	0.259	0.403	0.421	0.053
Gender	0.093	0.293	0.566	**0.010**	0.060	**0.002**	**0.021**	**<0.0005**	**0.004**	**0.002**
SW Ex	**0.001**	**0.165**	0.090	0.061	0.700	0.975	0.890	0.935	0.594	0.704
MS	0.071	0.359	0.811	0.075	0.237	0.194	0.839	0.099	0.141	0.193
Job type	0.096	**<0.05**	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	**<0.05**	**<0.0005**

BMI, body mass index; **DHQ1**, appetite disruption question; **DHQ2**, avoidance of certain foods to prevent stomach upsets question; **DHQ3**, nauseous question; **DHQ4**, heartburn question; **DHQ5**, digestion difficulties question; **DHQ6**, bloated stomach or flatulence question; **DHQ7**, abdominal pain question; **DHQ8**, constipation or diarrhoea question; **TDHS**, total digestive health score; **SW Ex**, shift work experience; **MS**, marital status.

3.3 RESULTS

3.3.1 Body mass index and leisure-time physical activity

An ANCOVA, adjusting for age, indicated that BMI was significantly associated with LTPA group ($P=0.047$; Table 3.3). Simple planned contrasts indicated a significant difference in BMI between LTPA group 1 (28.4±5.4 $kg \cdot m^2$) and group 2 only (24.6±2.8 $kg \cdot m^2$; $P=0.002$). Differences between LTPA group 1, 3 and 6 approached statistical significance ($P<0.095$).

Table 3.3. Unadjusted and adjusted associations of leisure-time physical activity with body mass index and total digestive health score.

	Leisure-time physical activity group						Unadjusted	Adjusted
	1	2	3	4	5	6	P-value	P-value
BMI	28.4±5.4	24.6±2.8	27.0±4.5	26.2±4.0	27.1±3.0	26.2±3.2	**0.013**	**0.047***
TDHS	16.6±5.4	16.2±5.4	15.8±6.0	15.3±4.6	13.9±4.6	14.4±5.3	0.332	0.836[§]

BMI (kg/m^2), body mass index; TDHS, total digestive health score; *, adjusted for age; [§], adjusted for gender and job type.

3.3.2 Digestive health and leisure-time physical activity

An ordinal regression analysis, adjusting for shift work experience and job type indicated that LTPA was not significantly related to appetite disruption frequency (Table 3.5). Similarly, another model, adjusting for age provided no evidence of an association between frequency of avoiding certain foods to prevent stomach upsets and LTPA (Table 3.5). Evidence was present for an association between frequency of heartburn and LTPA; specifically, shift workers in the LTPA group 2 suffered less frequently from heartburn than individuals in LTPA group 6 (odds ratio: 0.31, 95% confidence interval, 0.12 to 0.83; Table 3.4). A relationship, adjusted for gender, was not present between frequency of nauseous, digestive difficulties, bloated stomach or flatulence, abdominal pain and LTPA (Table 3.5). Furthermore, models which adjusted for gender and job type provided no evidence of a relationship between frequency of constipation or diarrhoea (Table 3.5), total digestive health score (P=0.836; Table 3.3) and LTPA.

Table 3.4. Unadjusted odds ratios and 95% confidence intervals between leisure-time physical activity and frequency of digestive symptoms.

	Leisure-time physical activity group					
Question #	1	2	3	4	5	6
DHQ1	0.77	0.61	0.64	0.89	0.77	-
	(0.28 to 2.07)	(0.23 to 1.61)	(0.24 to 1.76)	(0.34 to 2.35)	(0.29 to 2.01)	
DHQ2	0.46	0.71	0.97	0.52	0.85	-
	(0.17 to 1.26)	(0.27 to 1.88)	(0.35 to 2.68)	(0.23 to 1.68)	(0.32 to 2.28)	
DHQ3	0.74	0.90	1.07	0.60	2.31	-
	(0.27 to 2.06)	(0.33 to 2.45)	(0.38 to 3.03)	(0.22 to 1.63)	(0.77 to 6.94)	
DHQ4	0.53	**0.31**	0.78	0.69	0.91	-
	(0.19 to 1.43)	**(0.12 to 0.83)**	(0.29 to 2.12)	(0.26 to 1.85)	(0.34 to 2.41)	
DHQ5	0.44	0.70	1.01	0.85	2.04	-
	(0.16 to 1.19)	(0.26 to 1.85)	(0.37 to 2.77)	(0.31 to 2.28)	(0.73 to 5.73)	
DHQ6	1.06	0.97	0.91	0.85	1.18	-
	(0.39 to 2.85)	(0.91 to 2.55)	0.34 to 2.47)	0.32 to 2.29)	0.45 to 3.10)	
DHQ7	0.63	0.67	0.88	1.23	2.88	-
	(0.23 to 1.71)	(0.25 to 1.76)	(0.32 to 2.41)	(2.24 to 3.39)	(0.96 to 8.59)	
DHQ8	**0.36**	0.57	**0.33**	0.60	1.26	-
	(0.13 to 0.99)	(0.21 to 1.54)	**(0.12 to 0.42)**	(0.22 to 1.64)	(0.45 to 3.50)	

DHQ1, appetite disruption question; **DHQ2**, avoidance of certain foods to prevent stomach upsets question; **DHQ3**, nauseous question; **DHQ4**, heartburn question; **DHQ5**, digestion difficulties question; **DHQ6**, bloated stomach or flatulence question; **DHQ7**, abdominal pain question; **DHQ8**, constipation or diarrhoea question.

Table 3.5 Adjusted odds ratios and 95% confidence intervals between leisure-time physical activity and frequency of digestive symptoms.

	Leisure-time physical activity group					
Question #	1	2	3	4	5	6
DHQ1*	1.08	0.99	0.86	1.15	1.06	-
	(0.55 to 2.13)	(0.50 to 1.94)	(0.46 to 1.66)	(0.60 to 2.21)	(0.56 to 1.99)	
DHQ2§	0.49	0.60	1.21	0.47	0.77	-
	(0.18 to 1.38)	0.22 to 1.64)	(0.42 to 3.45	(0.20 to 1.31)	(0.28 to 2.09)	
DHQ3♦	0.98	1.13	1.27	0.69	1.21	-
	(0.34 to 2.83)	(0.39 to 3.18)	(0.44 to 3.70)	0.25 to 1.90)	(0.75 to 6.85)	
DHQ4	NA	NA	NA	NA	NA	-
DHQ5♦	0.60	0.93	1.27	0.98	2.04	-
	(0.21 to 1.69)	(0.34 to 2.55)	(0.46 to 3.56)	(0.36 to 2.66)	(0.73 to 5.77)	
DHQ6♦	1.58	1.30	1.11	0.98	1.15	-
	(0.56 to 4.43)	0.48 to 3.52)	(0.40 to 3.06)	(0.36 to 2.68)	(0.44 to 3.05)	
DHQ7♦	0.79	0.91	1.10	1.56	2.80	-
	(0.34 to 2.78)	0.33 to 2.51)	(0.39 to 3.09)	(0.55 to 4.43)	(0.93 to 8.47)	
DHQ8+	0.67	1.00	0.48 (0.17 to	0.83	1.37	-
	(0.23 to 1.98)	(0.34 to 2.95)	1.37)	(0.29 to 2.37)	(0.48 to 3.96)	

DHQ1, appetite disruption question; **DHQ2**, avoidance of certain foods to prevent stomach upsets question; **DHQ3**, nauseous question; **DHQ4**, heartburn question; **DHQ5**, digestion difficulties question; **DHQ6**, bloated stomach or flatulence question; **DHQ7**, abdominal pain question; **DHQ8**, constipation or diarrhoea question. *, adjusted for age and job type; §, adjusted for age; ♦, adjusted for gender; +, adjusted for gender and job type.

3.4 DISCUSSION

This is the first study of the relationship of LTPA with BMI and digestive symptoms in shift workers. It has been shown for the first time that LTPA is inversely associated with BMI and positively related to frequency of heart burn in shift workers, although there was no evidence of dose-response relationships. Despite this, these original

findings indicate no association between LTPA and the frequency of the other digestive symptoms in shift workers.

The finding of an inverse relationship between LTPA and BMI is consistent with most of the literature. Unfortunately, a cross-sectional study design cannot determine whether excessive adiposity is cause or consequence of low LTPA. Measurement of physical activity objectively in prospective studies has not produced compelling evidence that physical inactivity causes obesity (American Institute for Cancer Research, 2007). Thus, it has been suggested that excessive energy intake, rather than physical inactivity (and thus low energy expenditure), is the cause of excess adiposity (Westerterp, 2008). Interestingly, recent studies have shown that acute bouts of physical activity may reduce energy intake by suppressing the orixgenic hormone acylated ghrelin and therefore hunger (Broom et al., 2007, 2009). The inverse relationship between LTPA and excess adiposity may be due to excess fat which inhibits LTPA. Support for this postulation comes from an experiment which showed that objectively measured free-living walking distance was reduced in lean and obese individuals after they had increased their body mass (Levine et al., 2008).

Surprisingly, BMI was only statistically significantly higher in LTPA group 1 when compared to LTPA group 2 and therefore a dose-response relationship was not observed, unlike other studies reviewed (Ross and Janssen, 2001). This finding is difficult to interpret and explain but may be due to measurement error. For example, LTPA levels, body mass and height were all self-reported and this probably introduced some error into the statistical model used. Future researchers should objectively measure the abovementioned factors, using a prospective study, in order to elucidate clearly whether a relationship exists between LTPA and BMI in shift workers.

The finding of a positive relationship between LTPA and frequency of heartburn in shift workers is in agreement with studies that have shown that an acute bout of physical activity causes the aforementioned symptom, possibly by inducing gastro-oesophageal acid reflux (Clark et al., 1989). However, Peters et al. (1999b) reduced running induced gastro-oesophageal acid reflux by administering omeprazole, but heartburn was still caused by the bout of physical activity. Thus, the importance of gastro-oesophageal acid reflex in physical activity mediated heartburn is probably weak. Factors such as non-acid gastro-oesophageal reflux, musculoskeletal problems and mechanical distension of the oesophagus from the stomach may also cause heartburn (Simren, 2002). Shift workers who suffer from heartburn, should avoid activities with high bodily agitation (e.g., running) and consuming food in the hour before undertaking physical activity (Clark et al., 1989).

No relationship was found between LTPA and frequencies of appetite disruption, avoidance of certain foods to prevent stomach upsets, nauseous, digestion difficulties, bloated stomach or flatulence, abdominal pain, constipation or diarrhoea in shift workers. Previous cross-sectional and experimental work involving only day time workers has mainly focused upon physical activity and constipation. Some researchers have reported that physical activity is inversely related to constipation (Donald et al., 1985, Sandler et al., 1990, Dukas et al., 2003, Daley et al., 2008), whereas others have observed no relationship (Klauser et al., 1992, Meshkinpour et al., 1998, Tuteja et al., 2005). On balance, the available evidence indicates, but far from conclusively, that physical activity may decrease frequency of constipation in day workers. This view is supported by an experiment which showed that a bout of physical activity reduces mouth-to-anus transit time (Oettle, 1991), possibly due to physical activity mediated alterations in gut blood flow, hormones

(e.g., motilin), neurogenics or mechanical bouncing of gastrointestinal contents (Peters et al., 2001). These possible mechanisms may not be relevant to shift workers because the etiology of their constipation is possibly primarily due to disruption of circadian rhythms (e.g., intestinal motor activity) rather than factors such as an inadequate intake of fibre seen in day workers (Vener et al., 1989, Hsieh, 2005). Therefore this may explain why this study did not find an inverse relationship between LTPA and constipation.

Nausea, bloating, abdominal pain, flatulence and diarrhoea are reported during and/or after physical activity in some individuals (Brouns and Beckers, 1993, Peters et al., 1999a). However, these reports are from individuals that undertake high amounts of physical activity (e.g., marathon runners and ultra-endurance athletes). It is unlikely that many shift workers in the sample were undertaking the amount of training required in order to compete in marathons or ultra-endurance races. Thus, this might explain this why study found no relationship between LTPA and the abovementioned digestive symptoms.

As highlighted above, LTPA was self-reported and so were digestive symptoms. The self-reporting of these variables probably introduced some measurement error into the ordinal regression models used in this study which may be the reason why LTPA was not associated with the majority of digestive symptoms. Future work should measure LTPA objectively and recruit a larger sample size in order to reduce the risk of a Type II error occurring.

3.5 CONCLUSION

This study has shown for the first time that an association between LTPA and BMI and frequency of heartburn is present in shift-workers whilst controlling for

confounding variables. However, LTPA does not appear to be related to other digestive symptoms in shift-workers. Future researchers should aim to improve upon this study by collecting objective measures of LTPA, body mass, height and using randomised control trials. Such an approach would be able to better elucidate the relationships between LTPA, BMI and digestive health in shift-workers. Moreover, exploration of the acute effects of physical activity on appetite control (gut and non-gut derived regulators) in shift-workers is another related area of research which should be addressed.

CHAPTER 4

ACUTE EFFECTS OF EVENING EXERCISE ON APPETITE RELATED FACTORS DURING A SIMULATED NIGHT-SHIFT WHILST A 'TYPICAL' FEEDING SCHEDULE IS USED

4.1 INTRODUCTION

Shift-work is a significant risk factor for body mass gain (Suwazono et al., 2008), which is probably explained by behavioural- and biological-based disruption of factors involved in energy balance (Atkinson et al., 2008). For example, circadian disruption has been shown to decrease circulating concentrations of the anerexoic hormone, leptin (Scheer et al., 2009). Shift-workers also tend to 'graze' on food during the night-shift (Reinberg et al., 1979, Reeves et al., 2004) when postprandial thermogenesis is lowest (Romon et al., 1993). Intuitively, a reduction in nocturnal feeding, via some intervention which can alter hunger during a night-shift, could help maintain energy balance and, therefore, benefit the long-term health of the shift-worker. One such intervention could be exercise.

Acylated ghrelin and leptin act on the hypothalamus to control energy balance by increasing (Wren et al., 2001a) and decreasing (Halaas et al., 1995) preferred food intake, respectively. It is known that an acute bout of non-exhaustive exercise lasting ≤90 min transiently suppresses plasma acylated ghrelin concentration (Broom et al., 2007, 2009), but generally has little affect upon plasma/serum leptin level (Kraemer et al., 1999a, Weltman et al., 2000, Fisher et al., 2001, Hulver and Houmard, 2003). These findings relate to the fact that exercise has been found to decrease hunger transiently post-exercise (Thompson et al., 1988, King et al., 1994, King and Blundell, 1995, Westerterp-Plantenga et al., 1997, Broom et al., 2007, 2009), although no change (King et al., 1997) or increases (Lluch et al., 1998, Maraki et al., 2005) in hunger have also been reported. These data indicate that acute bouts of exercise may support maintenance of energy balance, not just via an increase in energy expenditure but in helping to regulate energy intake. Nevertheless, all previous related research has been undertaken during the hours of daylight on

participants living a 'normal' diurnal existence. No previous researcher has examined the effects of prior evening exercise on appetite regulation when awake throughout the night. Given the knowledge that circadian rhythms exist in human metabolism (Ribeiro et al., 1998, Simon et al., 2000, Holmback et al., 2002, 2003a, 2003b) and in the physiological responses to exercise (Drust et al., 2005), it is questioned whether the exercise-mediated responses of hunger, plasma acylated ghrelin and serum leptin are different when awake and eating during a simulated night-shift compared with the findings from previous studies undertaken during the day.

4.2 METHODS

4.2.1 Participants

In previous experiments (Broom et al., 2007, 2009), exercise has been found to mediate relatively large (26-35%) decreases in plasma acylated ghrelin concentration, which can be measured with good within-assay precision (CV=4.8-6.6%). The primary comparison in this study was the difference in plasma acylated ghrelin averaged over the simulated night-shift between the exercise and no exercise trials. Using the above estimations of effect size and variance, it was estimated that a sample size of 6 would result in adequate statistical power (>80%) so that the predicted magnitude of difference changes would be statistically significant in this repeated-measures experiment. Therefore, 6 healthy males were recruited (mean±SD: age = 30±8 yrs, height = 178±8 cm, BMI = 23.1±1.1 $kg \cdot m^2$, peak oxygen uptake = 49±7 $ml \cdot kg^{-1} \cdot min^{-1}$). Participants lived a conventional diurnal lifestyle prior to the experiment (nocturnal sleep of 6-8 $h \cdot day^{-1}$) and provided written informed consent to participate. None of the participants had been involved in night-work before or travelled across more than 2-3 time-zones in the previous 6 months. The study was approved by the local ethics committee.

4.2.2 Preliminary measurements

Before the first laboratory visit, participants refrained from exercise as well as consumption of alcohol and caffeine for 24 h. Participants were instructed to consume 5 ml·kg^{-1} body mass of water, and nothing else, in the 2 h before arrival. After height and body mass were measured (Seca Ltd, Birmingham, UK), participants completed a continuous incremental test on a cycle ergometer (Ergo_bike, 8000 TRS, Daum Electronics, Fürth, Germany) to determine their peak oxygen uptake ($\dot{V}O_{2peak}$), which was measured with an automated gas analyzer (MetaMax, Cortex Biophysik, Leipzig, Germany). The first stage of the $\dot{V}O_{2peak}$ protocol consisted of cycling at 100 Watts for 2 min. Exercise intensity was then increased by 25 Watts every 2 min until the participants reached volitional fatigue. Immediately before exercise began, heart rate was measured using short-range radio telemetry (Polar S610i; Polar Electro Oy, Kempele, Finland) and was continuously monitored and recorded at 2 min intervals, and at the point of exhaustion. Participants were deemed to have reached $\dot{V}O_{2peak}$ if one or more of the following criteria were met: a plateau in $\dot{V}O_2$ (<2.1 ml·kg^{-1}·min^{-1}), a respiratory exchange ratio of 1.15 or above, and/or a heart rate within 10 beats·min^{-1} of a participant's age-predicted maximum heart rate.

4.2.3 Experimental procedures

Approximately one week after undertaking the $\dot{V}O_{2peak}$ test, participants performed the first of two experimental trials which were administered in a random order and were separated by ≥7 days. Before both trials, participants abstained from exercise and the consumption of alcohol and caffeine for ≥48 h. Participants were instructed to sleep between 23:00 and 07:00 h the night before each trial. On arrival at the

laboratory, participants did not report any atypical sleep characteristics (e.g. number of nocturnal awakenings and perceived sleep latency) during this pre-test night. The stipulated bedtimes were within ± 1 h of the participants 'normal' times of retiring and rising. Both trials began at 19:00 h and continued until 05:00 h in a laboratory where mean (±SD) ambient temperature was 22°C (±0.3), relative humidity was 47% (±4) and light intensity was 200 lux. Participants weighed and recorded their food and drink intake, using a diary, during the two days that preceded their first trial, and replicated it before their second trial.

On the day of each trial, participants reported to the laboratory at 17:30 h, after a 7.5 h fast (this was confirmed by questioning the participant about their food and drink intake in the previous 7.5 h). Thereafter, participants promptly adopted a semi-supine position and a cannula was inserted into an antecubital vein. Before both trials began, participants consumed a test meal at 18:00 h and consumed it within 15 min, then rested quietly. At the start of the exercise trial between 19:00-20:00 h, participants cycled at 50% $\dot{V}O_{2peak}$ (individual absolute power outputs ranged from 130-170 W) on the ergometer described above. Participants completed self-paced mental and physical work at 21:00-21:05 h, 00:00-00:05 h and 05:00-05:05 h in order to simulate activities that may occur during a typical night-shift. At 22:00 h and 02:00 participants consumed two isocaloric test meals. Participants consumed 100 ml of water at 18:00 h and thereafter at 1 h intervals until 05:00 h. At all other times when participants were not completing tasks, eating or providing blood samples, they rested quietly (e.g., sat watching television). The control trial was identical to the intervention trial, except participants rested (e.g., sat whilst reading) quietly instead of exercising between 19:00 and 20:00 h.

4.2.4 Test meals

The test meal consumed at 18:00 h has been utilized previously (Broom et al., 2007). The meal consisted of a sandwich (Cheddar cheese, mayonnaise and butter), crisps, a chocolate bar and milk-shake. The meal provided 1.47 g carbohydrate, 0.34 g protein, 0.81 g fat and 60 kJ per kg body mass. The test meals consumed at 22:00 and 02:00 h were identical to the abovementioned meal in content, but were halved in portion size. This feeding schedule was chosen to replicate 'typical' food intake of night workers (Reinberg et al., 1979, Reeves et al., 2004).

4.2.5 Perceived hunger and activity

Perceived hunger was measured using a validated scale (Broom et al., 2009), ranging from 0 (not hungry) to 15 (very hungry). Perceived hunger was recorded at 1-h intervals between 19:00 and 05:00 h. Participant's activity was recorded via an accelerometer (Actiwatch AW4; Cambridge Neurotechnology Ltd, Cambridge, UK), attached at the wrist. Data were recorded at 19:00 h until 05:00 h using 10 sec epocs.

4.2.6 Blood sampling

In both trials, and after participants had been in a semi-supine position for at least 10 min, blood samples (10 ml) were collected into syringes at 19:00 h (immediately before the exercise) and thereafter at 1 h intervals until 05:00 h, via a cannula placed in an antecubital vein. At 20:00 h, in both trials, (i.e., at the end of exercise in the intervention trial) blood was collected immediately after participants had adopted the semi-supine position. In order to maintain cannula patency, 5 ml of non-heparinised saline was flushed through the system after each sampling point. Moreover, 2-3 ml

of blood/saline was drawn off at the beginning of each sampling point to prevent a high concentration of saline being present in the samples.

At each sampling point, blood was immediately dispensed into serum separator tubes, pre-cooled lithium heparin and EDTA (some of which contained *p*-hydroxymercuribenzoic acid to prevent the degradation of acylated ghrelin) tubes. Lithium heparin and EDTA (not containing *p*-hydroxymercuribenzoic acid) tubes were placed in a refrigerated centrifuge (4°C) within 15 min of collection, and spun at 4000 revs/min for 15 min. Serum separator tubes, after standing at room temperature for 30-35 min were placed into a centrifuge and spun, as detailed above. Immediately after collection, EDTA tubes containing *p*-hydroxymercuribenzoic acid were placed into a refrigerated centrifuge (4°C) and spun for 10 min at 3500 revs·min^{-1}. The resultant supernatant was dispensed into a microtube, and 1 M hydrochloric acid was then added (100 uL per mL of plasma) and the sample was subsequently spun for 5 min at 3500 revs·min^{-1} in a refrigerated centrifuge (4°C). Immediately after centrifuging, plasma/serum supernatants were dispensed into microtubes and stored at -80°C for later analysis. Acylated ghrelin and non-esterified fatty acids (NEFA) concentrations were determined from plasma derived from EDTA tubes. Leptin and insulin levels were measured in serum. Glucose and triglyceride concentrations were determined from plasma derived from lithium heparin tubes. At each sampling point, hemoglobin concentraion and hematocrit percentage were determined in order to estimate plasma volume changes (Dill and Costill, 1974).

4.2.7 Biochemistry

Plasma acylated ghrelin concentrations were determined by ELISA (SPI BIO, Montigny le Bretonneux, France) with within- and inter-assay CVs of 14.0% and 1.7%, respectively. Serum leptin and insulin concentrations were determined by Muliplexing, and had within- and inter-assay CVs of 59.9%, 57.6%, 6.6% and 7.1%, respectively. Plasma concentrations of glucose, triglyceride and non-esterified fatty acids were determined via enzymatic, colorimetric methods (Randox Laboratories LTD, Cumlin, Northern Ireland) with within-assay variations of 1.3%, 1.3% and 3.6%. Accuracy was monitored with quality control sera (Randox Laboratories LTD, Cumlin, Northern Ireland) and samples from each participant were analysed in the same batch to prevent inter-assay variation.

4.2.8 Statistical analysis

Paired-samples *t*-tests or Wilcoxon signed ranked tests (if the paired differences did not follow a Guassian distribution) were used to determine if baseline values were significantly different between trials. No significant differences at baseline were found for all but one of the outcome variables studied ($P \geq 0.17$). Therefore, the post-exercise data for hormones and metabolites were analysed with two factor (trial x time) repeated measures linear mixed models (Cnaan et al., 1997). Post-exercise data for activity counts were analysed with a paired-samples *t*-test The *P*-value for the baseline differences in mean hunger rating approached significance ($P=0.081$). Therefore, an analysis of covariance using baseline values from both trials as covariates was used to analyse this particular outcome variable (Vickers and Altman, 2001). Plasma volume changes were not significantly different between trials, thus unadjusted values for metabolites and hormones were analysed and are reported.

All statistical procedures were performed via SPSS for Windows version 15 (SPSS, Inc., Chicago, USA). Descriptive data are presented as means±SD, unless otherwise stated.

4.3 RESULTS

Mean plasma acylated ghrelin concentration was 86.5±40.8 pg/ml during the night-shift (20:00-05:00) after exercise compared with 71.7±37.7 pg/ml in the control trial (95% confidence interval for trial main effect = 2.9 to 26.7 pg/ml, P=0.015; Figure 4.1A). During the night-shift, serum leptin concentration was also significantly higher in the exercise (263±242 pg/ml) than control trial (187±221 pg/ml; P=0.017; Figure 4.1B). No significant interactions between trial and time were found for acylated ghrelin and leptin (P>0.05).

Throughout the night-shift, serum insulin concentration was significantly higher when preceded by exercise (193±127 pg/ml) compared with rest (128±98 pg/ml; P=0.001; Figure 4.2A) and plasma triglyceride concentration was also significantly higher in the exercise trial (1.8±0.7 mmol/L) than control (1.6±0.6 mmol/L; P=0.004; Figure 4.2B). Similarly, NEFA concentration was significantly higher when preceded by exercise (0.36±0.16 mmol/L) compared with rest (0.26±0.14 mmol/L; P<0.0005). Plasma glucose was 5.8±0.9 mmol/L during night-work in the exercise trial and 5.5±1.0 mmol/L in the rest trial, the differences between trials not reaching statistical significance (P=0.090; Figure 4.2C).

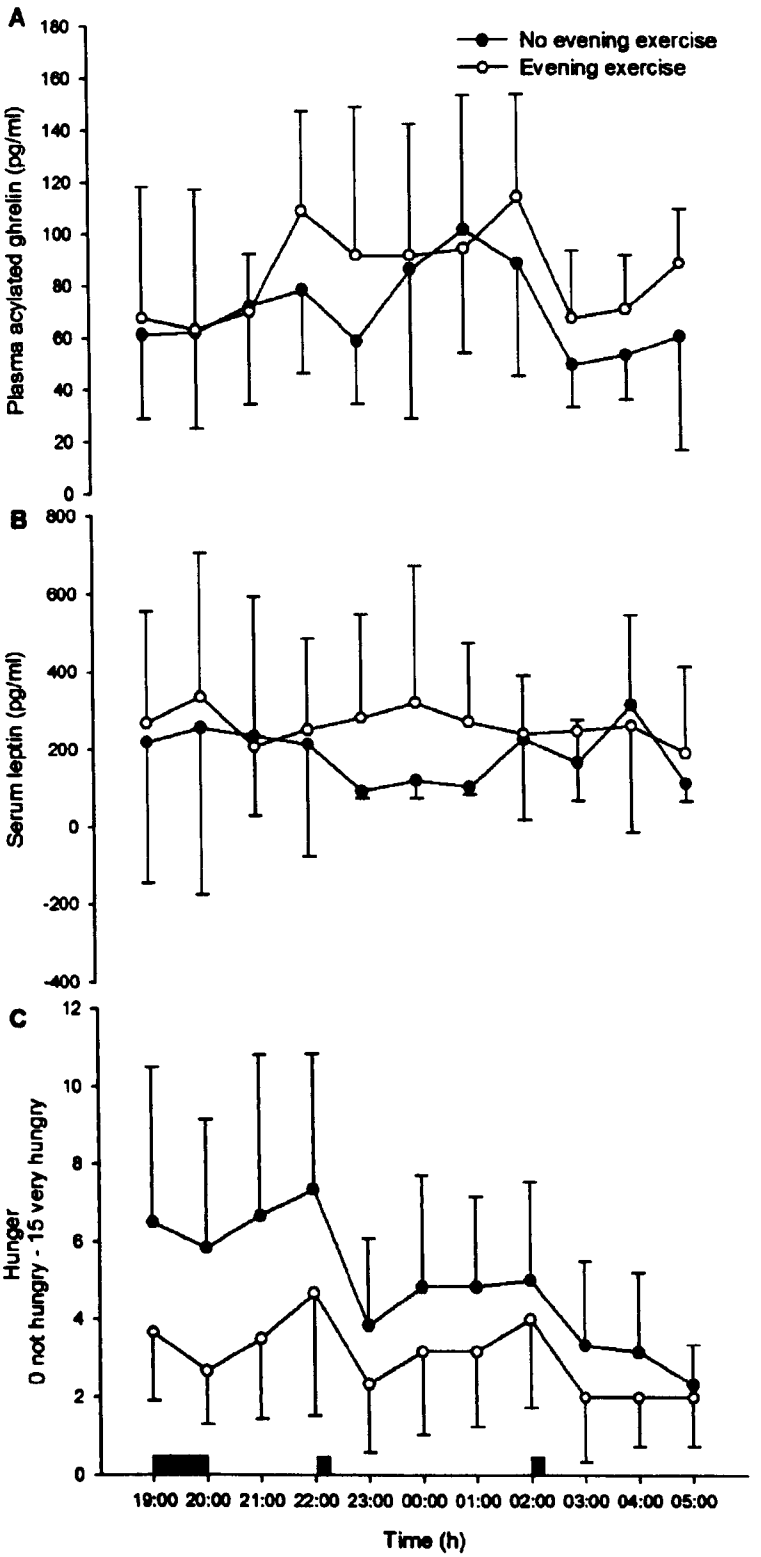

Figure 4.1. Circulating acylated ghrelin (**A**) and leptin (**B**) concentrations and hunger (**C**) during exercise and control trials (mean±SD). *Solid rectangle, cycling; grey square, test meal consumption.*

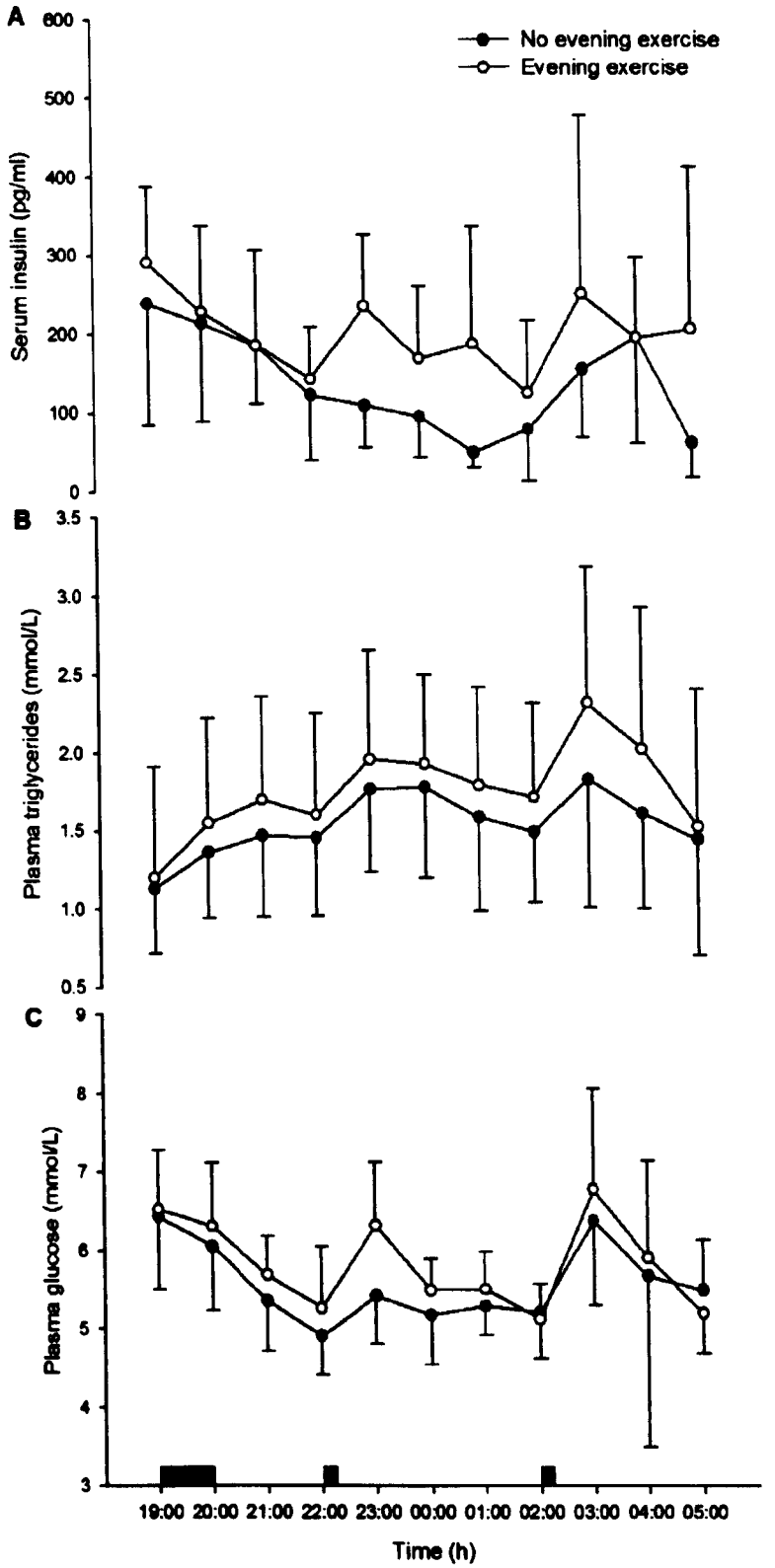

Figure 4.2. Circulating insulin (**A**), triglyceride (**B**) and glucose (**C**) concentrations during exercise and control trials (mean±SD). *Solid rectangle, cycling; grey square, test meal consumption.*

Night-shift activity counts were significantly higher in the exercise (263±139) than control trial (178±104; $P<0.0005$). Mean hunger during the night-shift generally reduced as meals were ingested throughout the night-shift (Figure 4.1C), but a significant interaction between trial and time was found ($P=0.015$). With the baseline values of hunger added as covariates, hunger was found to decrease more rapidly over time during the night-shift when no even exercise had been undertaken. There was no significant effect of trial on hunger ($P=0.120$; Figure 4.1C).

4.4 DISCUSSION

No previous researcher has published data regarding appetite regulation during nocturnal waking after a bout of prior exercise in the evening. The novel findings of this study are that an early-evening bout of exercise mediated higher values of circulating concentrations of acylated ghrelin and leptin during a subsequent night-shift compared to no prior exercise. These findings contrast with those from similar investigations undertaken during the daytime (Weltman et al., 2000, Broom et al., 2007, 2009) and suggest that the appetite-suppressant influences of exercise that has generally been observed diurnally are absent when that exercise is taken during the evening prior to a period of night-work.

It is known that intravenous infusions of glucose (Nakagawa et al., 2002), insulin (Mohlig et al., 2002) and NEFA (Gormsen et al., 2006) suppress total circulating ghrelin levels. In this study, there was no significant difference in plasma glucose concentration between trials, although circulating levels of insulin and NEFA were found to be higher nocturnally after prior exercise. These findings offer evidence that the relationships between these metabolic- and appetite-related outcomes change at night, when energy intake is controlled. Ghrelin secretion is also known to be regulated by somatostatin, growth hormone, melatonin, glucagon,

parasympathetic nervous activity and thyroid hormones (van der Lely et al., 2004). Melatonin might be a focus of further study, given its known role in the circadian system and the fact that it is responsive to exercise (Atkinson et al., 2003).

Researchers have reported that acute intravenous infusion of insulin increases circulating leptin concentrations (Malmstrom et al., 1996, Utriainen et al., 1996), whereas others have observed no effect (Dagogo-Jack et al., 1996, Ryan and Elahi, 1996). In this study, the exercise-mediated increase in nocturnal serum insulin concentration occurred concurrently with increased serum leptin level. Intravenous infusion of milk (98% triglycerides) significantly induces leptin resistance at the blood-brain barrier (Banks et al., 2004). In this study, triglyceride concentration was significantly higher during the night-shift when preceded by evening exercise than rest. Besides this finding having significant health implications itself (Gill, 2004), it may explain why leptin concentration was higher in the exercise than control trial. Circulating leptin levels are affected by factors other than insulin and triglycerides, such as glucocorticoids and the sympathetic nervous system (Trayhurn et al., 1998). Cortisol is a glucocorticoid which increases post-exercise (Kanaley et al., 2001). Circulating levels of leptin increase in response to cortisol (Dagogo-Jack et al., 2005, Laferrere et al., 2006). In this study, evening exercise may have increased circulating level of cortisol which subsequently raised serum leptin concentration. Furthermore, post-exercise serum cortisol response exhibits a circadian variation, with a larger response occurring in the night compared to morning (Kanaley et al., 2001). This time of day effect may explain why this investigation observed an increase in post-exercise serum leptin instead of no response communicated in several day time studies (Weltman et al., 2000, Fisher et al., 2001).

The nocturnal level of plasma acylated ghrelin was approximately 21% higher during the night-shift that followed exercise compared with control. Circulating concentrations of the anorectic hormones leptin and insulin (Woods et al., 1984) were also higher after exercise. Therefore, little evidence of a reciprocal relationship between acylated ghrelin and leptin was found in this study on post-exercise nocturnal responses. Hunger was found to decrease less rapidly during the night-shift when evening exercise had been undertaken, which may suggest the exercise-modulated increase in leptin (a hormone which increases preferred food intake) was less influential on the hunger ratings than the increase in acylated ghrelin. Nevertheless, it is noted that despite the attempts to control for baseline differences in hunger with an ANCOVA model, two of the participants remained relatively hungry after consuming the test meal at 18:00 h in the non-exercise trial. Consequently, this may have accentuated the decrease in hunger after eating the test meal at 22:00 h in the no exercise condition. It seems that the effects of exercise on hunger are also inconsistent with daytime studies, with increases (Lluch et al., 1998, Maraki et al., 2005) and decreases (Thompson et al., 1988, King et al., 1994, King and Blundell, 1995, Westerterp-Plantenga et al., 1997, Broom et al., 2007, 2009) in hunger being reported after exercise. The exercise intensity used in this study was lower than that chosen by other investigators who have reported post-exercise hunger suppression, and this could be an important factor. Nevertheless, it was deemed important to select a moderate rather than a high exercise intensity that was feasible for night-workers to adopt.

There are several limitations to this study, firstly in terms of the generalizability of data to real shift-workers. A high-fat, energy dense meal was administered in keeping with the previous daytime-based studies on the acylated ghrelin responses

to exercise (Broom et al., 2007, 2009). Although some shift-workers tend to eat high-fat, energy dense foods on the night-shift, it is possible that some shift-workers consume meals with different macronutrient and energy content, and therefore the results presented above may not be applicable to all shift-workers. The participants in this study were not permanent night-workers. Therefore the findings from this study may have been different if participants had been studied over a number of night-shifts. Nevertheless, Folkard (2008) concluded that very few (<3%) fixed night-workers exhibit full circadian adjustment and therefore the findings from this study may be indeed relevant to these permanent workers as wells as those who switch from days to nights frequently.

Under conditions of controlled diet but only partially-controlled activity, prior exercise was found to increase wrist activity measured during the night-shift. Therefore, it is unclear whether, in real shift-work circumstances, the overall increase in energy expenditure modulated by an exercise bout may or may not cause a negative energy balance when night-workers are completely free to choose their diet. Future researchers in this area might measure energy expenditure via more valid methods (e.g., wearing an accelerometer on the waist rather than the wrist) under conditions of 'free-living' whilst measuring food intake during the night-shift.

As in almost all exercise-related experiments of this type, it was extremely difficult to blind participants as to which experimental trial they were undertaking before arriving to the laboratory. Thus, it is possible that the expectation of exercising may have influenced hunger values in this study, but it unlikely to have impacted upon the biological blood-borne data. A future study might involve a comparison of different exercise intensities which may help reduce such an expectancy effect.

This experiment is novel, it involved participation in exercise as well as rigorous longitudinal physiological monitoring (including cannulation) when awake during the night. Therefore, only a relatively small sample of young non-shift-working men participated. Nevertheless, relatively large and statistically significant effects of prior exercise on the primary outcome (i.e. main effect of trial on plasma acylated ghrelin concentration) and some secondary outcomes were found, and so a Type II error and any associated concerns about statistical power are not as relevant to the primary and many secondary findings. However, it is acknowledged that this study could have been underpowered to detect significant interactions between trial and time for some of the outcome variables. Statistical power is relevant only in terms of a Type II error, i.e. when differences between trials were not significant. Statistical significance is also an indicator that effect sizes are large relative to error variance. The error variance in this repeated measures study is defined by the test-retest CV (rather than the between-subjects variability indicated by the error bars in the Figures above). The CVs were relatively small for most of the study outcomes.

Finally, it is recognised that exercise may induce a phase-shift to some circadian rhythms, such as melatonin and core body temperature (Atkinson et al., 2007). In this study, it is possible that evening exercise caused a phase-shift in the outcome variables and this could explain the findings. However, it was difficult to recruit participants in a multi-day study so that enough data for circadian rhythm description were obtained. Any future attempt at this would need to carefully control for the masking effects of being awake at night.

4.5 CONCLUSION

No previous researcher has examined the effects of prior exercise on appetite regulation when participants are awake at night. This situation was researched given its application to a change from day- to night-work. A prior bout of early evening exercise was found to have no effect on mean hunger scores but increases circulating levels of acylated ghrelin and leptin during a simulated night-shift compared with no prior exercise. In previous research, participants exercised during the hours of daylight (usually mid-morning) and contrasting results were generally reported. These data indicate that time of day is an important factor to consider in the exploration of relationships between exercise, metabolism and appetite in order to explain why shift-work is a risk factor for long-term weight-gain.

CHAPTER 5

ACUTE EFFECTS OF EVENING EXERCISE ON APPETITE RELATED FACTORS DURING A SIMULATED NIGHT-SHIFT WHILST AN 'ATYPICAL' FEEDING SCHEDULE IS USED

5.1 INTRODUCTION

The main findings from Chapter 4 were that an acute bout of moderate intensity evening exercise increases circulating concentrations of acylated ghrelin and leptin during a simulated night-shift when a typical feeding schedule (i.e., grazing on small meals) is adopted (Reinberg et al., 1979, Reeves et al., 2004). This feeding schedule is adopted by many night-workers due to canteen facilities not being available and therefore there is a reliance of purchasing snacks from vending machines (Lennernas et al., 1995). In some large organisations, it is likely that adequate canteen facilities are available during the night which may allow the night-worker to have one large meal midway through the work period in the same way as a day-worker would have lunch rather than multiple smaller meals or snacks.

Meal frequency has a significant effect on postprandial metabolic and hormonal responses. For example, some researchers have demonstrated, using an isocaloric and iso-macronutrient design, that consuming food frequently rather than occasionally decreases mean postprandial circulating concentrations of insulin, growth hormone and NEFAs in individuals (Jenkins et al., 1990, 1992, Wolever, 1990, Bertelsen et al., 1993), whereas other researchers have not observed such results (Solomon et al., 2008). Insulin (Mohlig et al., 2002), NEFA (Gormsen et al., 2006) and possibly growth hormone (Cappiello et al., 2002, Freda et al., 2003) are important regulators of acylated ghrelin. Insulin (Malmstrom et al., 1996, Utriainen et al., 1996) is also known to be involved in the control of leptin secretion.

Therefore, it is possible that meal frequency can influence postprandial concentrations of acylated ghrelin and leptin, although this theory has not been formally explored in a night-work context. Moreover, meal frequency may interact with the effects that an acute bout of exercise has upon circulating concentrations of acylated ghrelin and leptin. Therefore, the aim of the study detailed in the present

chapter is to examine the effects of an acute bout of evening exercise upon circulating levels of acylated ghrelin and leptin during a simulated night-shift in which one large meal is consumed.

5.2 METHODS

5.2.1 Participants

As detailed in the previous chapter, approximately 6 participants are required to provide adequate statistical power (>80%) in order to detect statistically significant differences between two trials. Thus, 7 healthy males (mean±SD: age = 32±8 yrs, height = 182±6 cm, BMI = 23.0±1.3 $kg \cdot m^2$, peak oxygen uptake = 50±7 $ml \cdot kg^{-1} \cdot min^{-1}$) who were living a conventional diurnal lifestyle (nocturnal sleep of 6-8 $h \cdot day^{-1}$) were recruited. Participants had not been involved in night-work before or travelled across more than 2-3 time-zones in the previous 6 months. Five of the participants recruited in this study were involved in the experiment described in Chapter 4. Participants provided written informed consent and this study was approved by the local ethics committee.

5.2.2 Preliminary measurements

These measurements are detailed in the previous chapter.

5.2.3 Experimental procedures

The experimental procedure is the same as detailed in the previous chapter, except for one part. Instead of consuming two small test meals at 22:00 h and 02:00 h, participants ingested one large test meal at 22:00 h.

5.2.4 Test meals

The test meal consumed at 18:00 h is as described in Chapter 4. The test meal consumed at 22:00 h was a combination of the two small test meals (consumed at 22:00 h and 02:00 h) described in the previous chapter. This feeding schedule was implemented to simulate a frequency of food intake of that is atypical for night-workers but typical (i.e. a reasonably large lunch) for day-workers (Reinberg et al., 1979, Reeves et al., 2004).

5.2.5 Perceived hunger and activity

Hunger and activity were measured according to the protocols detailed in the previous chapter.

5.2.6 Blood sampling

Blood was drawn as detailed in Chapter 4.

5.2.7 Biochemistry

Hormones and metabolites were determined as described in the previous chapter. Plasma acylated ghrelin had within- and inter-assay CVs of 13% and 8%, respectively. Serum leptin and insulin had within- and inter-assay coefficient variations of 25%, 21%, 10% and 12%, respectively. Plasma concentrations of glucose, triglyceride and NEFA had within-assay variations of 2%, 3% and 4%. In regard to the aforementioned metabolites, samples from each participant were analysed in the same batch to prevent inter-assay variation.

5.2.8 Statistical analysis

Paired-samples *t*-tests or Wilcoxon signed ranked tests (if the paired differences did not follow a Guassian distribution) were used to determine if baseline values were significantly different between trials. No significant differences at baseline were found for the outcome variables studied ($P \geq 0.22$). Therefore, the post-exercise data for hormones and metabolites were analysed with two factor (trial x time) repeated measures linear mixed models (Cnaan et al., 1997). Post-exercise data for activity counts were analysed with a paired-samples *t*-test. Plasma volume changes were not significantly different between trials, thus unadjusted values for metabolites and hormones were analysed and are reported. All statistical procedures were performed via SPSS for Windows version 15 (SPSS, Inc., Chicago, USA). Descriptive data are presented as means±SD.

5.3 RESULTS

Mean plasma acylated ghrelin concentration was 101.2±77.3 pg/ml during the night-shift (20:00-05:00 h) after exercise compared with 102.4±100.6 pg/ml in the control trial ($P=0.862$; Figure 5.1A). During the night-shift, serum leptin concentration was significantly higher in the exercise (142±139 pg/ml) than control trial (120±94 pg/ml; $P=0.003$; Figure 5.1B). No significant interactions between trial and time were found for acylated ghrelin or leptin ($P>0.05$).

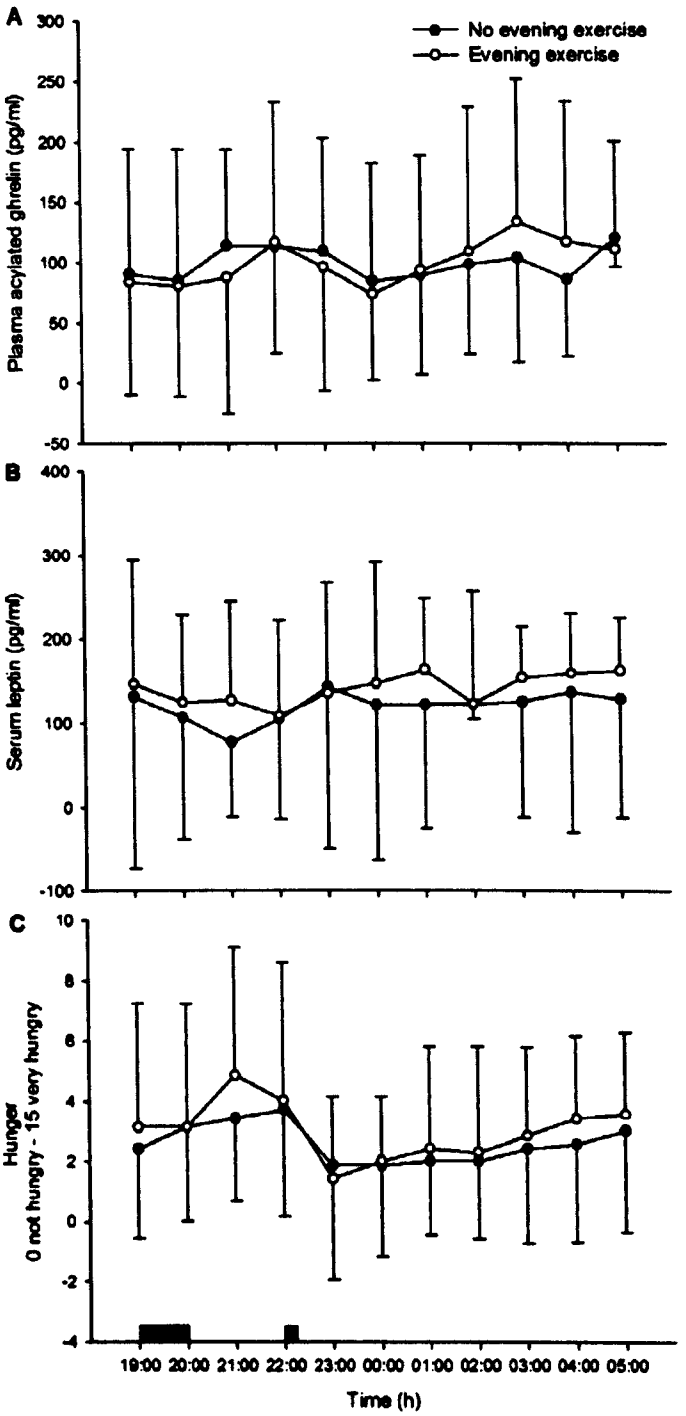

Figure 5.1. Circulating acylated ghrelin (**A**) and leptin (**B**) concentrations and hunger (**C**) during exercise and control trials (mean±SD). *Solid rectangle, cycling; grey square, test meal consumption.*

Throughout the night-shift, serum insulin concentration was not significantly different when preceded by exercise (135±109 pg/ml) compared with rest (141±139 pg/ml; P=0.683; Figure 5.2A). An interaction between trial and time in regard serum insulin concentration approached statistical significance (P=0.051); insulin was found to decrease more rapidly over time (20:00-05:00 h) during the night-shift when evening exercise had been undertaken (Figure 5.2A). During the night-shift, plasma triglyceride concentration was not significantly different in the exercise trial (2.4±1.9 mmol/l) than control trial (2.1±1.4 mmol/l; P=0.288; Figure 5.2B). Plasma NEFA concentration was significantly higher when preceded by exercise (0.48±0.18 mmol/l) compared with rest (0.33±0.17 mmol/l; P<0.0005). Plasma glucose was 5.8±0.9 mmol/l during night-work in the exercise trial and 5.6±0.7 mmol/l in the rest trial (P=0.390; Figure 5.2C). No significant interactions between trial and time were found for triglyceride, NEFA or glucose (P>0.05).

Night-shift activity counts were significantly higher in the exercise (220±43) than control trial (125±31; P=0.001).During the night-shift, mean hunger was not significantly different when preceded by exercise (3.0±3.4 units) compared to rest (2.6±2.8 units; P=0.643; Figure 5.1C). No significant interaction between trial and time was present in regard to hunger (P=0.840; Figure 5.1C).

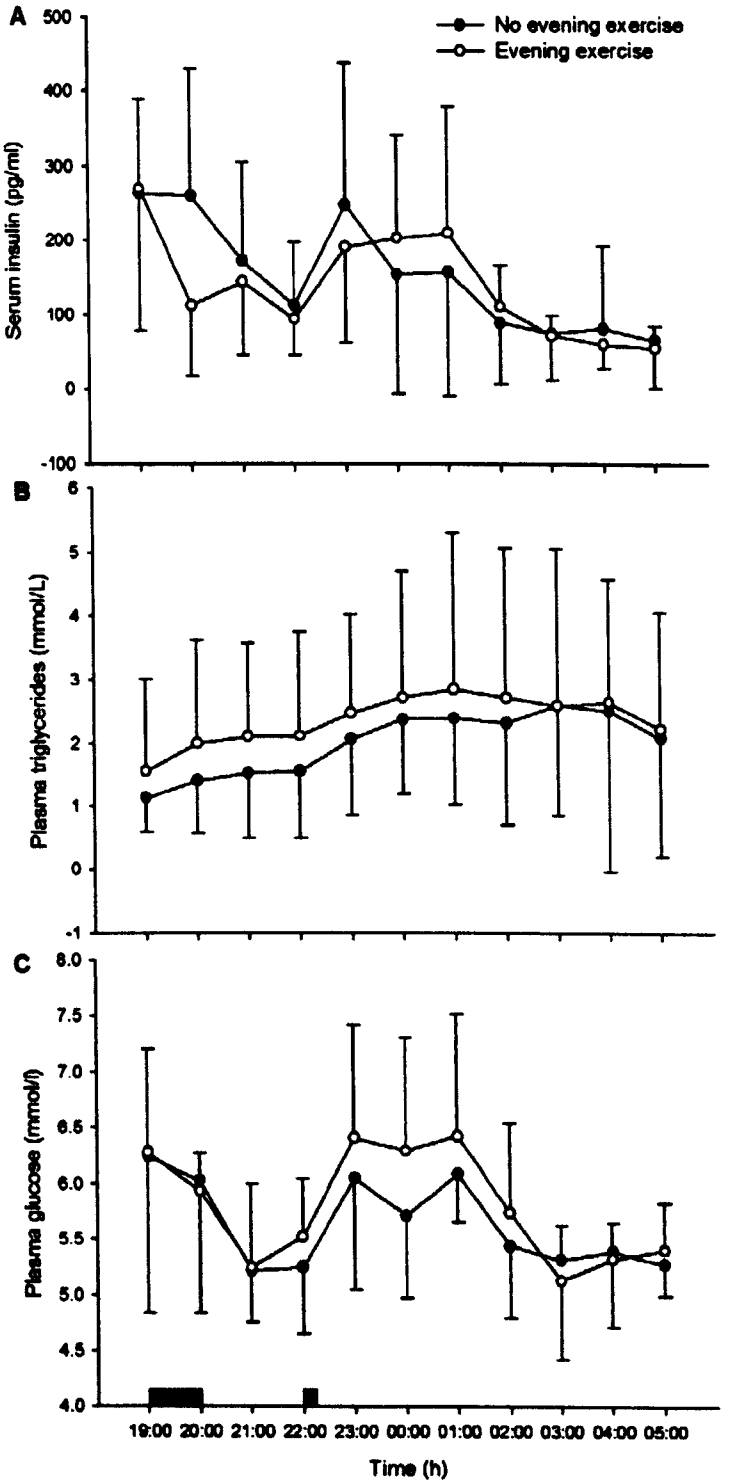

Figure 5.2. Circulating insulin (**A**), triglyceride (**B**) and glucose (**C**) concentrations during exercise and control trials (mean±SD). *Solid rectangle, cycling; grey square, test meal consumption.*

5.4 DISCUSSION

The results of this study indicate that during a simulated night-shift in which one large test meal is consumed, prior evening exercise does not affect plasma acylated ghrelin concentrations, but increases serum leptin levels. In the previous study involving ingestion of two smaller meals, circulating concentrations of both acylated ghrelin and leptin were increased by exercise. Taken together, these findings suggest that the influence of exercise upon acylated ghrelin during a simulated night-shift is dependent on nocturnal meal frequency.

Circulating total ghrelin concentration is suppressed by intravenous infusions of insulin (Mohlig et al., 2002) and NEFA (Gormsen et al., 2006). Moreover, acromegalic patients have lower levels of circulating total ghrelin than healthy individuals (Cappiello et al., 2002, Freda et al., 2003), indicating that growth hormone suppresses the aforementioned orexigenic. Mean postprandial circulating levels of the abovementioned suppressors of total ghrelin are significantly higher when meal frequency is low compared to high (e.g., two large meals vs. six smaller meals; Jenkins et al., 1990, 1992, Wolever, 1990, Bertelsen et al., 1993). Despite this, Solomon et al. (2008) reported that meal frequency was not related to postprandial plasma concentrations of total ghrelin; however, the measurement of total ghrelin may have masked changes in circulating levels of acylated ghrelin. Thus, although not formally examined, it is possible that meal frequency has an effect upon circulating levels of acylated ghrelin. The mean relative postprandial plasma concentrations of insulin, NEFA and growth hormone may have been higher in this study which utilised two test meals compared to three in Chapter 4. Circulating concentrations of insulin, NEFA and growth hormone are likely to be more powerful regulators of acylated ghrelin than physical activity, this may explain why night-shift

plasma acylated ghrelin level was unaffected by prior exercise in this chapter unlike in Chapter 4.

Circulating concentrations of NEFA were significantly higher during the night-shift when evening exercise had been undertaken compared to rest and this response was observed in Chapter 4 too. As briefly described above, increasing circulating NEFA concentration via intravenous infusion of intralipid/heparin whilst clamping blood concentrations of some known regulators of total ghrelin (e.g., glucose, insulin, growth hormone) significantly suppresses total serum ghrelin concentration (Gormsen et al., 2006). Despite the aforementioned finding, in this study, the significant increase in plasma NEFA concentration observed during the exercise trial did not suppress plasma acylated ghrelin concentration, perhaps indicating that the regulatory effects of NEFA are only related to des-acylated ghrelin. Moreover, it is possible that another factor which increases circulating levels of total ghrelin was also higher during the night-shift preceded by prior exercise compared to rest and this may have counteracted the suppressive effects of NEFA.

In this study, serum leptin levels were significantly higher during the night-shift when preceded by evening exercise compared to rest and this finding mirrors that of Chapter 4. Increasing circulating levels of insulin (Malmstrom et al., 1996, Utriainen et al., 1996) and triglycerides (Banks et al., 2004) has been reported to increase serum concentrations of leptin. However, in this study, mean circulating levels of insulin and triglycerides during the simulated night-shift were not significantly affected by prior evening exercise. Thus, between trial differences in the abovementioned factors cannot explain why an increase in night-shift serum leptin level was observed in the exercise trial. Factors other than insulin and triglycerides affect circulating leptin levels; such factors include glucocorticoids and the

sympathetic nervous system (Trayhurn et al., 1998). A commonly known glucocorticoids is cortisol, this hormone is known to increase after exercise (Kanaley et al., 2001) and increases circulating levels of leptin (Dagogo-Jack et al., 2005, Laferrere et al., 2006). Therefore, in this study, it is possible that evening exercise mediated an increase night-shift cortisol levels which in turn increased circulating levels of leptin. Post-exercise serum cortisol response is dependent upon time of day, with a greater response occurring in the night compared to morning (Kanaley et al., 2001). This diurnal variation may explain why this study observed an increase in post-exercise leptin level rather than no effect reported in many day time investigations (Fisher et al., 2001, Weltman et al., 2000).

It is evident from Figure 5.1A,B and 4.1A,B (see Chapter 4) that circulating levels of acylated ghrelin and leptin did not differ significantly between experimental conditions until food was consumed. This indicates that food consumption frequency *per se* is an important factor which mediates post-exercise responses of circulating levels of acylated ghrein (in Chapter 4 only) and leptin.

Night-shift plasma acylated ghrelin concentration was unaffected by prior evening exercise. However, nocturnal serum leptin level was significantly higher in the exercise than control trial. Thus, a reciprocal relationship between circulating levels of acylated ghrelin and leptin was present in this study, unlike in Chapter 4. Consequently, one may think that night-shift hunger would be lower in the exercise than control trial. However, hunger was similar in the exercise and control trial. There are many possible reasons for the findings relating to acylated ghrelin and leptin not translating into a decrease in night-shift hunger when preceded by evening exercise. Firstly, the hunger questionnaire used in this study may not be able to detect very subtle changes. Secondly, participants were in a controlled laboratory environment

and this may interfere with their true feelings of hunger. Thirdly, there are other hunger related hormones (some that have probably yet to be discovered) which were not measured, it is possible that changes in such hormones (e.g., a decrease in peptide YY_{3-36}) counteracted the effect that evening exercise had upon circulating levels of leptin (Batterham et al., 2003).

Several limitations of the previous study (Chapter 4) are also applicable to this chapter. These limitations will not be discussed here (see section 4.4 in Chapter 4) and thus not repeated, but relate to the recruitment of non-night-workers, macronutrient content of test meals, statistical power, night-shift physical activity and the possibility of exercise causing a phase-shift in some outcome variables. A new, notable limitation is that the participants in this and the previous chapter were different and therefore this limits the certainty one can say that the effect of evening exercise upon night-shift plasma acylated ghrelin level is dependent on meal frequency. However, it should be noted that some participant characteristics were very similar in this (mean±SD: age = 32±8 yrs, height = 182±6 cm, BMI = 23.0±1.3 $kg \cdot m^2$, peak oxygen uptake = 50±7 $ml \cdot kg^{-1} \cdot min^{-1}$) and the previous chapter (mean±SD: age = 30±8 yrs, height = 178±8 cm, BMI = 23.1±1.1 $kg \cdot m^2$, peak oxygen uptake = 49±7 $ml \cdot kg^{-1} \cdot min^{-1}$).

5.5 CONCLUSION

This investigation is the first to examine the effect of evening exercise on appetite regulation during a night-shift whilst an 'atypical' feeding schedule is undertaken. Evening exercise did not affect plasma acylated ghrelin concentration but increased serum leptin levels during the night-shift. Somewhat surprisingly, these responses did not cause a reduction in mean hunger in the exercise trial. The lack of an

exercise-mediated effect upon night-shift plasma acylated ghrelin concentration in this study contrasts from the related finding in Chapter 4. This indicates that meal frequency is an important factor to consider in the exploration of relationships between evening exercise and night-shift appetite regulation.

CHAPTER 6

WITHIN-SUBJECT RELATIONSHIPS BETWEEN POST-EXERCISE CONCENTRATIONS OF ACYLATED GHRELIN, LEPTIN AND OTHER VARIABLES RELATED TO HUMAN METABOLISM AND HUNGER

6.1 INTRODUCTION

Many researchers have investigated the mechanisms in which circulating levels of ghrelin and leptin are regulated at rest. Data from such investigations have indicated that intravenous infusion of insulin (Mohlig et al., 2002), glucose (Nakagawa et al., 2002) and NEFA (Gormsen et al., 2006) decrease circulating level of ghrelin at rest. Broom et al. (2007, 2009) investigated the relationship between circulating concentrations of metabolites, hormones and acylated ghrelin during and post-exercise and found that circulating insulin and glucose level did not correlate with plasma acylated ghrelin concentration at any time-point. This finding suggests that the exercise-mediated suppression of ghrelin observed by Broom et al. (2007, 2009) was not mediated by alterations in circulating insulin and glucose level. It is feasible that exercise alters circulating acylated ghrelin via altering other metabolites such as NEFA and triglyceride. Nevertheless, this postulation has never been explored.

Circulating leptin level has been found to be affected by insulin (Malmstrom et al., 1996, Utriainen et al., 1996), glucose (Levy and Stevens, 2001), cortisol (Dagogo-Jack et al., 1996, Laferrere et al., 2006), NEFA (Garcia-Lorda et al., 2003, Stefan et al., 2001) and triglyceride (Banks et al., 2004) at rest. Researchers have also examined the relationship between leptin and some of the abovementioned factors as well as others (e.g., insulin-like growth factor) during and after acute short-term (<60 min) exercise (Fisher et al., 2001, Jurimae and Jurimae, 2005, Elias et al., 2000). Fisher et al. (2001) reported that a hierarchical regression model including the predictors; circulating levels of insulin, glucose and cortisol accounted for 98% of the variation in serum leptin level during and after exercise. Fisher et al. (2001) observed no relationship between circulating level of leptin and NEFA during or post-exercise. Elias et al. (2000) and Jurimae and Jurimae (2005) reported a positive association

between insulin-like growth factor and leptin post-exercise. To date, no researcher has determined if the post-exercise response of serum leptin concentration is associated with plasma triglyceride level or indeed hunger.

The primary aims of this chapter were: (i) determine if post-exercise changes in plasma acylated are related to circulating levels of NEFA and triglyceride and (ii) establish whether post-exercise response of serum leptin concentration is associated with plasma triglyceride level and hunger. Investigating such aims provides information regarding how post-exercise circulating levels of acylated ghrelin and leptin are regulated. The abovementioned aims were achieved by pooling participant data from Chapters 4 and 5. This subsequently provided a sample size of 13 males and therefore superior statistical power than if the analysis had been undertaken using unspooled data from Chapters 4 and 5.

6.2 METHODS

6.2.1 Participants

Participants were recruited as detailed in Chapters 4 and 5. The participants recruited for Chapters 4 and 5 were pooled to provide a sample of 13 healthy males (mean±SD: age = 31±8 yrs, height = 180±8 cm, BMI = 23.1±1.2 $kg \cdot m^2$, peak oxygen uptake = 49±7 $ml \cdot kg^{-1} \cdot min^{-1}$).

6.2.2 Preliminary measurements

These measurements are detailed in Chapters 4 and 5.

6.2.3 Experimental procedures

The experimental procedures are detailed in Chapters 4 and 5.

6.2.4 Test meals

Test meals are described in Chapters 4 and 5.

6.2.5 Perceived hunger

Hunger was measured according to the protocol detailed in Chapters 4 and 5.

6.2.6 Blood sampling

Blood was drawn as detailed in Chapters 4 and 5.

6.2.7 Biochemistry

Hormones and metabolites were determined as described in Chapters 4 and 5.

6.2.8 Statistical analysis

Within-subjects correlations were calculated using the appropriate statistical modelling approach described by Bland and Altman (1995) to explore relations between the changes in the different variables over time (exercise trial only; 20:00-22:00 h). Specifically, the two outcomes variables were circulating levels of acylated ghrelin and leptin and the predictor variables were circulating concentrations of insulin, glucose, triglyceride, NEFA and perceived hunger. All statistical procedures were performed via SPSS for Windows version 15 (SPSS, Inc., Chicago, USA). Statistical significance was set at $P<0.05$.

6.3 RESULTS

6.3.1 Acylated ghrelin

The within-subject correlation between post-exercise values of acylated ghrelin and insulin was r=-0.38 (Figure 6.1; P=0.052). Statistically significant correlations between acylated ghrelin, glucose and hunger were also observed (Figure 6.2 & 6.3; Table 6.1). No significant correlations between acylated ghrelin and triglyceride, NEFA or leptin were present (Table 6.1).

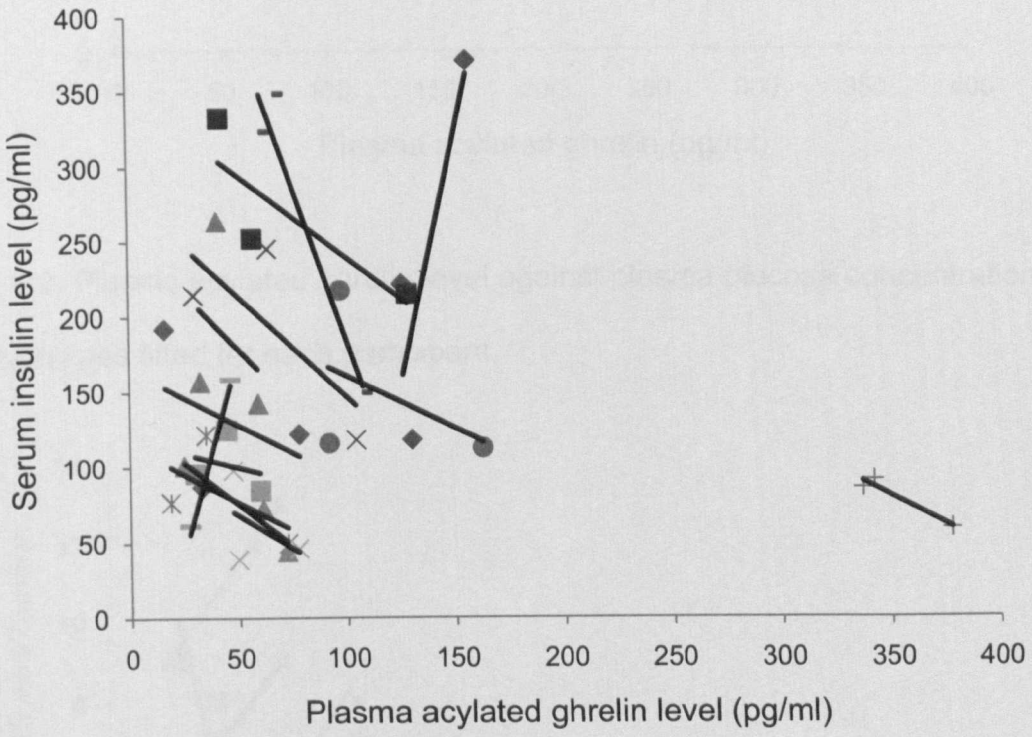

Figure 6.1. Plasma acylated ghrelin level against serum leptin concentration, with regression lines fitted for each participant.

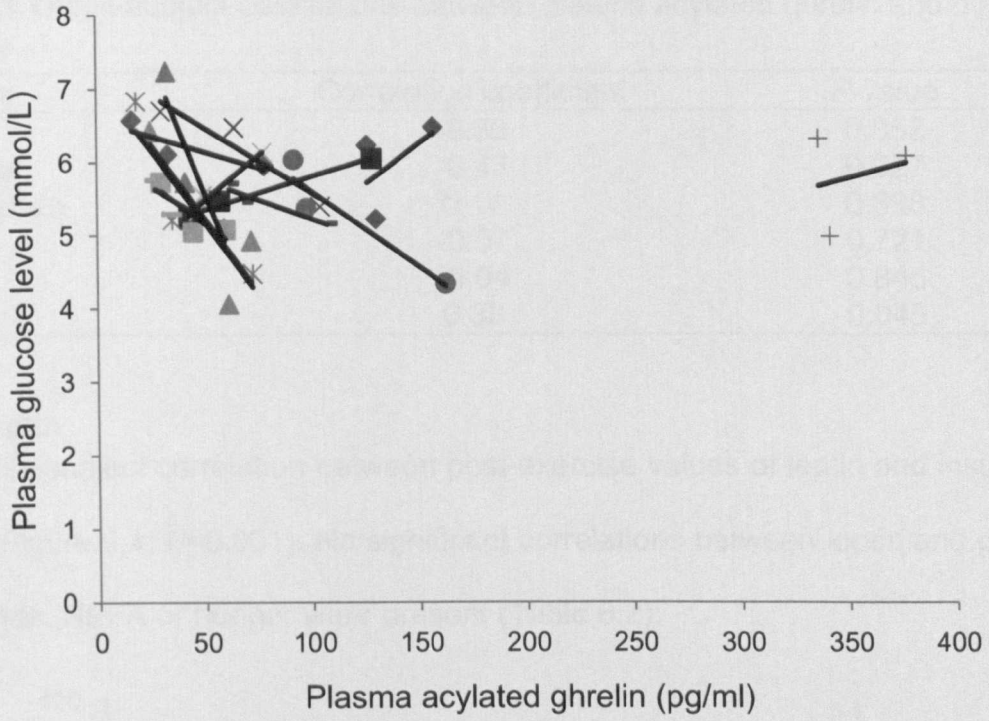

Figure 6.2. Plasma acylated ghrelin level against plasma glucose concentration, with regression lines fitted for each participant.

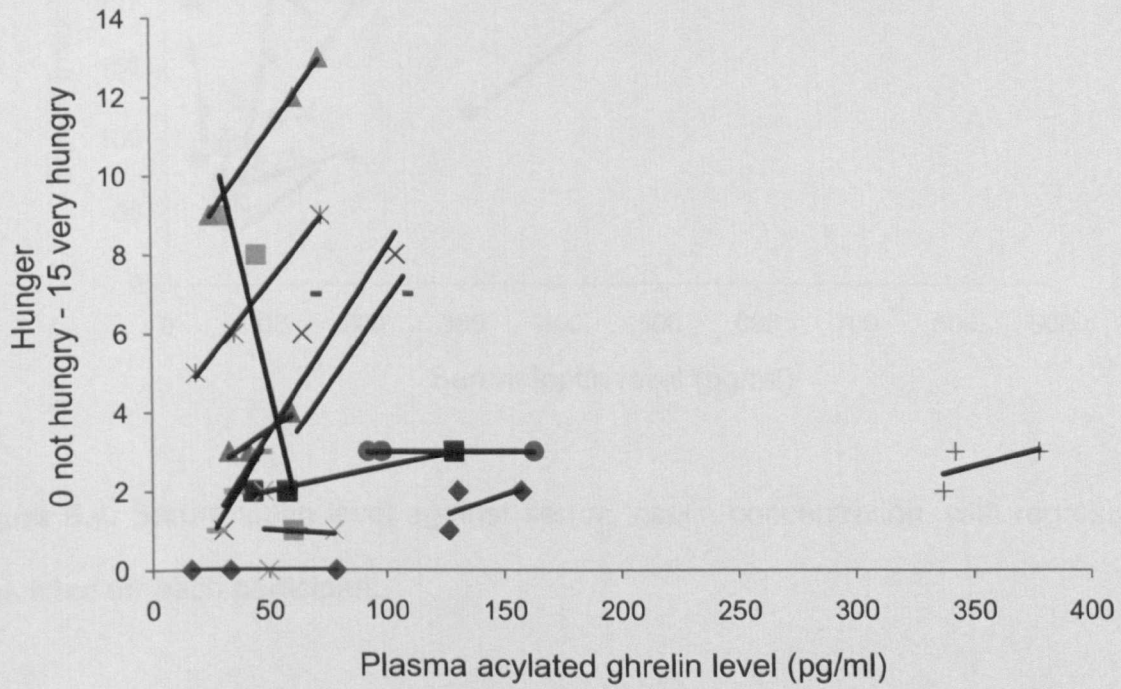

Figure 6.3. Plasma acylated ghrelin level against hunger ratings, with regression lines fitted for each participant.

Table 6.1 Within-subject correlations between plasma acylated ghrelin and other variables

Variable	Correlation coefficient	*P*-value
Insulin	-0.38	0.052
Glucose	-0.43	0.027
Triglyceride	0.17	0.398
NEFA	-0.07	0.721
Leptin	-0.04	0.845
Hunger	0.38	0.045

6.3.2 Leptin

The within-subject correlation between post-exercise values of leptin and insulin was r=0.61 (Figure 6.4; P=0.001). No significant correlations between leptin and glucose, triglyceride, NEFA or hunger were present (Table 6.2).

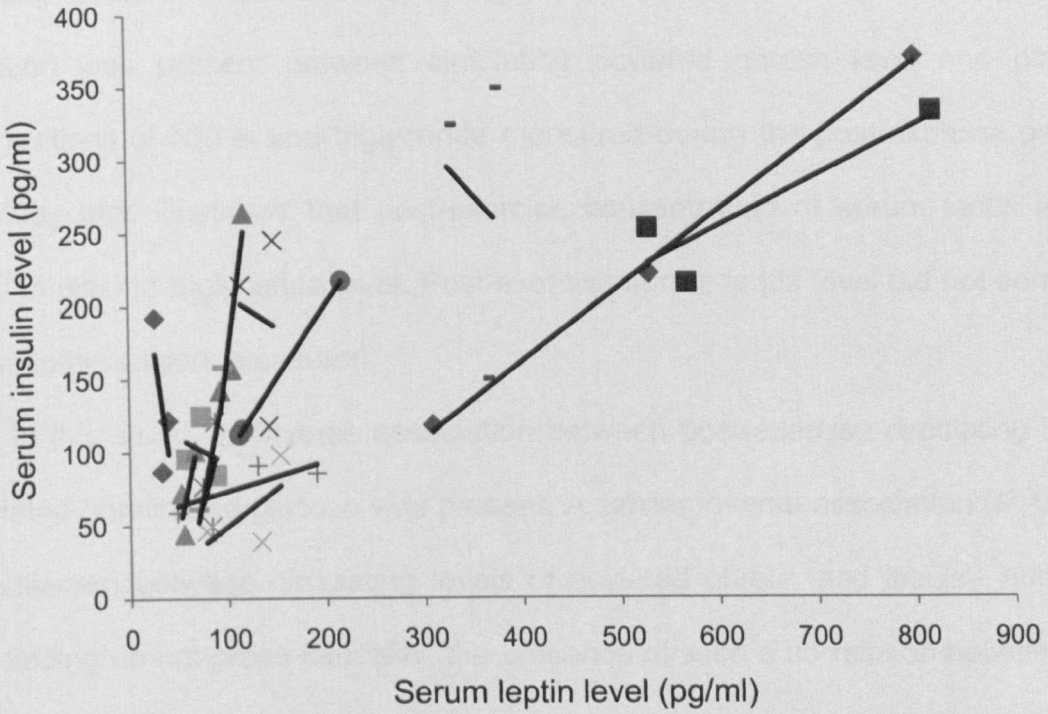

Figure 6.4. Serum leptin level against serum insulin concentration, with regression lines fitted for each participant.

Table 6.2 Within-subject correlations between serum leptin and other variables

Variable	Correlation coefficient	*P*-value
Insulin	0.61	0.001
Glucose	0.09	0.664
Triglyceride	-0.25	0.200
NEFA	0.13	0.516
Acylated ghrelin	-0.04	0.845
Hunger	0.01	0.957

6.4 DISCUSSION

This is the first study to show that post-exercise circulating concentrations of acylated ghrelin are inversely correlated over time with plasma glucose concentrations. Furthermore, there was evidence of an inverse association between circulating levels of insulin and acylated ghrelin measured over time. No significant correlation was present between circulating acylated ghrelin level and plasma concentrations of NEFA and triglyceride measured during the post-exercise period. This study also illustrates that post-exercise concentration of serum leptin is not related to plasma triglyceride level. Post-exercise serum leptin level did not correlate with any other factors measured.

In this study, an inverse association between post-exercise circulating levels of acylated ghrelin and glucose was present. A similar inverse association (P=0.052) was observed between circulating levels of acylated ghrelin and insulin. Although these finding do not prove causality, the presence of such a correlation between two outcomes is a necessary condition for causality to be present. Previous research has not involved the formal correlation approach that was adopted in the present study, and data from these past experiments have been conflicting. Ghrelin has been found to increase glucose (Broglio et al., 2001, Broglio et al., 2003) while it has also been reported that ghrelin is suppressed by glucose (Kim et al., 2007). Similarly, experiential studies demonstrate that ghrelin increases insulin (Broglio et al., 2003)

but is decreased by higher insulin levels (Mohlig et al., 2002). Broom et al. (2007, 2009) reported no association between plasma levels of glucose and acylated ghrelin which contrasts with the data in the present study. Between studies differences in regard to exercise intensity utilised and time of day (early morning versus evening) that exercise occurred may explain the conflicting findings. Indeed, at rest, circulating ghrelin concentration is higher in the day time than at night (Natalucci et al., 2005) and the opposite occurs in regard to serum leptin level (Perfetto et al., 2004).

In this study, no association between post-exercise circulating levels of acylated ghrelin and NEFA was present. Thus, one may hypothesise that the suppressive effect of NEFA on circulating ghrelin levels at rest (Gormsen et al., 2006) is not present after exercise. However, it should be noted that Gormsen et al. (2006) only measured total circulating ghrelin concentrations, thus it is possible that NEFA only suppress unacylated rather than acylated ghrelin at rest.

In the present study, no association between post-exercise circulating levels of acylated ghrelin and triglyceride was present. This finding is perhaps surprising considering there is evidence that triglyceride suppresses ghrelin secretion in humans (Feinle-Bisset et al., 2005). Nevertheless, a positive association between post-exercise circulating levels of acylated ghrelin and hunger was present in this study. This is an important finding and has been reported elsewhere (Broom et al., 2007, 2009), and it indicates that the relationship between hunger and acylated ghrelin is present after exercise as it is at rest (Wren et al., 2001a). Thus, an exercise-mediated increase of plasma acylated ghrelin level should concurrently increase hunger and this may induce body mass gain overtime in some individuals.

Despite this, in Chapter 4, evening exercise did not increase night-shift hunger although plasma acylated ghrelin level was significantly higher.

In this study, a positive association between post-exercise circulating levels of leptin and insulin was present. In keeping with the explanation above regarding acylated ghrelin, glucose and insulin, this issue is complicated further by experimental studies indicating that leptin increases (Ahren and Havel, 1999) and is suppressed by insulin (Malmstrom et al., 1996, Utriainen et al., 1996). After evaluating the experimental data that are currently available, it is difficult to speculate on the causality regarding post-exercise levels of leptin and insulin. Fisher et al. (2001) also reported a relationship between post-exercise circulating levels of leptin and insulin, whereas Jurimae and Jurimae (2005) observed no association. A reason for these conflicting findings is difficult to determine, but may be related to inter-study differences in regard to exercise intensity and duration, fitness status and nutritional status of participants.

In this chapter, neither post-exercise levels of glucose, NEFA or triglycerides correlated with serum level of leptin. This indicates that the regulatory effects of glucose (Levy and Stevens, 2001), NEFA (Garcia-Lorda et al., 2003, Stefan et al., 2001, Evans et al., 2001) and triglycerides (Banks et al., 2004) on circulating leptin level at rest are abolished post-exercise. The lack of a correlation between post-exercise circulating levels of NEFA and leptin in this study supports the related finding of Fisher et al. (2001).

No other researcher has assessed the relationship between circulating leptin level and hunger during or after exercise. No association between serum leptin level and hunger was present post-exercise in this study. This finding is interesting because if exercise decreases circulating leptin level, as reported by some

researchers (Elias et al., 2000, Jurimae and Jurimae, 2005, Legakis et al., 2004) but not all (e.g., Fisher et al., 2001, Weltman et al., 2000, Sari et al., 2007), it is unlikely to increase post-exercise hunger and thus food intake. This finding also supports the notion that leptin is a long-term regulator of energy balance (Klok et al., 2007). However, researchers have demonstrated that circulating leptin level responds acutely in response to food intake (Dallongeville et al., 1998, Havel, 2000), indicating that the hormone does have a role in short-term energy balance.

6.5 CONCLUSION

Plasma concentration of acylated ghrelin is related to circulating levels of insulin and glucose but not NEFA or triglyceride after exercise. Post-exercise, serum level of leptin is associated with circulating concentration of insulin but not glucose, NEFA or triglyceride. These findings indicate that the relationship between some regulators of acylated ghrelin and leptin at rest is altered post-exercise. Moreover, only circulating level of acylated ghrelin was associated with hunger post-exercise. This suggests that post-exercise concentrations of acylated ghrelin are more important than serum leptin levels in regard to hunger regulation.

CHAPTER 7

EFFECTS OF A LIFESTYLE INTERVENTION BASED ON MOTIVATIONAL INTERVIEWING ON SHIFT-WORKER'S ADIPOSITY AND DIGESTIVE HEALTH: A RANDOMISED CONTROLLED TRIAL

7.1 INTRODUCTION

As discussed in previous chapters, shift-work is a risk factor for excess adiposity and digestive symptoms. Findings from Chapter 3 indicated that there is a relationship between LTPA and BMI in shift-workers. This relationship has also been observed in many studies involving day-workers (e.g., Pitsavos et al., 2005). Nevertheless, the results from Chapter 3 indicated that LTPA did not moderate aspects of digestive health reported by shift-workers which contrasts with data from studies involving day-workers (Everhart et al., 1989, Colwell et al., 1998, Brown et al., 2000). Although the findings from Chapter 3 are useful, the study design was cross-sectional in nature and thus has limitations, especially in the causality. Such limitations encountered in cross-sectional studies can be avoided by implementing a randomised controlled trial.

Only two studies involving an apparent randomised controlled trial of a lifestyle intervention that is administered specifically to shift-workers have been published (Harma et al., 1988, Elliot et al., 2007). Harma et al. (1988) found that a 4-month supervised physical activity program did not change body mass and skinfold measurements. These authors noted a statistically significant decrease in gastrointestinal symptoms in individuals from the control group. Elliot et al. (2007) reported that motivational interviewing, which focussed upon increasing fire-fighter's physical activity level and improving dietary habits, significantly reduced body mass gain over time.

Although the abovementioned randomised controlled trials provide useful data applicable to shift-workers, only one of these studies (Elliot et al., 2007) utilised an individualised program. An individualised lifestyle program is possibly more appropriate than a rigid or supervised one for shift-workers because their work-rest

schedules are consistently altering. The study by Elliot et al. (2007) involved US fire-fighters. Such workers are likely to be already undertaking high amounts of physical activity compared to other shift-workers (e.g., police communications officers; see Fullick et al., 2009). Furthermore, in general, fire-fighters are allowed to sleep during the night-shift if not called out to an emergency. Consequently, the negative effects of nocturnal wakefulness might not apply as readily to fire-fighters as to call centre workers or nurses for example.

Motivational interviewing is a client-based counselling program which has been demonstrated to improve many long-term health outcomes in individuals (Burke et al., 2003). A recent meta-analysis concluded that motivational interviewing significantly reduces BMI to a greater extent than standard treatments in day-workers (Rubak et al., 2005). This finding probably relates to motivational interviewing increasing physical activity levels and/or improving dietary habits to a larger degree than standard treatments in some studies (Brodie and Inoue, 2005, Bennett et al., 2007, Elliot et al., 2007, Perry et al., 2007, Hardcastle et al., 2008), but not all (Bennett et al., 2008). Despite these encouraging findings, no controlled investigation into the efficacy of motivational interviewing for decreasing adiposity and gastrointestinal symptoms via changes in physical activity levels and dietary habits in UK shift-workers who do not require physical fitness for their job and remain awake during all night-shifts has been undertaken.

Thus, the primary aims of this study were to determine using a 12-week randomised controlled trial, involving UK shift-workers who do not require physical fitness for their job and remain awake during all night-shifts, the effect of physical activity and dietary centred motivational interviewing upon adiposity and digestive health.

7.2 METHODS

7.2.1 Participants and setting

Shift-workers were recruited from the Operational Communications Branch of Greater Manchester Police between December 2008 and February 2009, using posters and the organisation's monthly magazine for staff. Approximately 600 shift-workers were exposed to the posters and magazine. Initially, participants contacted the researcher and were emailed information about the project and eligibility. Exclusion criteria for this study were a clinically diagnosed disease (e.g., diabetes), aged <18 or >65 years, inability to be physically active and not been involved in shift-work (involving night-work) concurrently for at least 6 months. If shift-workers met the inclusion criteria and provided written informed consent, baseline data were obtained and then participants were assigned to either the control or intervention group using minimisation strategies (Altman and Bland, 2005). The allocation sequence was minimised to reduce any baseline differences between intervention and comparator groups in age, gender, shift-work experience and education level. All participants were involved in rapid, forward rotation shift-work which involved periods of night-work (workers were awake throughout this shift). All measurements and face-to-face contacts occurred at work-sites belonging to Greater Manchester Police. Ethical approval was granted by a local ethics committee.

7.2.2 Study design

A randomised controlled trial was conducted according to guidelines laid down in the CONSORT statement (Moher et al., 2001). The study included 2 groups; a comparator and intervention group.

7.2.3 Intervention group – motivational interviewing

Motivational interviewing is a directive, client- or participant-centred counselling style which aims to elicit behaviour change by helping clients explore and resolve ambivalence (Miller and Rollnick, 2002). Motivational interviewing is more focused and goal-orientated than non-directive counselling (Rollnick and Miller, 1995). Counsellors use a range of techniques (e.g., key questions and reflective listing) and styles (e.g., empathy) to increase an individual's motivation or readiness to change. Specifically, reflective listening is a key aspect of motivational interviewing, allowing clarification of goals, worries and inducing motives for change in the client's own words.

An important aspect of motivational interviewing is assisting individuals through their ambivalence regarding behaviour change. The client is encouraged to determine a part of their behaviour they would like to alter and discuss the benefits and barriers to such a change. If required, the counsellor facilitates the above process by helping the individual to think of mechanisms that would overcome difficulties. Moreover, the client sets realistic goals for behaviour change. The counsellor provides objective feedback in a neutral manor in order to anchor conversations regarding personal goals and highlight discrepancies between current behaviours and individual aspirations. Reflective comments are used when resistance and defensiveness is exhibited in order to prevent progress to behavioural change being damaged (Amrhein et al., 2003).

In this study, motivational interviewing sessions explored individual reasons for increasing physical activity levels and improving dietary habits. When client's requested information regarding physical activity and diet, the counsellor provided such information (see Joint British Societies', 2005, Haskell et al., 2007) and referred

the individual to the healthy lifestyle booklet previously provided during baseline measurements. Each shift-worker in the intervention group received 3 motivational interviewing sessions. The first session occurred in study week 1, was face-to-face, lasted approximately 30 minutes and occurred whilst the participant was at work. The remaining 2 sessions occurred in study weeks 5 and 9, were via telephone and lasted between 15-20 minutes. To serve as a reminder, the counsellor provided a form containing the participant's goals at the end of the face-to-face motivational interviewing session. The sessions were conducted by an individual trained in motivational interviewing.

7.2.4 Comparator group

Participants in the control group were not encouraged or discouraged from changing aspects of their lives. Shift-workers in this group did not have contact with the researchers at anytime, except for when measurements were obtained in study weeks 0 and 13. Whilst attending baseline measurements, the control group received an identical healthy lifestyle booklet to that provided to the intervention group.

7.2.5 Measurement protocol

Assessments were made by researchers un-blinded to experimental conditions. Measurements were recorded at baseline (0 weeks) and post-intervention (13 weeks). In an attempt to control for possible circadian variation in some outcome measures, participants attended each measurement session at the same time of day (within 1 hour), having fasted for approximately 8 hours.

On arrival, participants completed a physical activity and digestive health questionnaire, and provided information (only at baseline) regarding age, gender, shift-work experience, education level, marital status and number of children. Following this, body mass was measured without heavy items of clothing (e.g., shoes) to the nearest 0.01 kg with calibrated, digital scales. Then height was measurement without shoes to the nearest 0.5 cm using a portable stadiometer. BMI was calculated as body mass in kilograms divided by the square of height in meters. Thereafter, waist circumference was measured at the narrowest point between the costal margin and lilac crest and hip circumference was measured at its greatest protuberance. Waist-to-hip ratio was determined by dividing waist circumference by hip circumference. Finally, participants were given a food frequency questionnaire and pre-paid envelope. Shift-workers were asked to complete the food frequency questionnaire at home, due to it being time consuming (approximately 30 min) and return it within 1 week to the researcher via the pre-paid envelope.

7.2.6 Physical activity questionnaire

Physical activity was assessed using the long version of the International Physical Activity Questionnaire (IPAQ). This questionnaire has been demonstrated to be both valid and reliable (Craig et al., 2003, Hagstromer et al., 2006) . The self-administered questionnaire covers 4 areas of physical activity: work-related, transportation, housework/gardening and leisure-time activity. In all of the areas, information pertaining to number of days per week and time spent per day in both moderate and vigorous activity are recorded. Walking time is also recorded during transportation and leisure-time. Practical examples relating to activities of moderate and vigorous intensity are given.

Outcome measures from the IPAQ used were: total walking Metabolic Equivalent of Task (MET)-min·week^{-1}, total moderate intensity activity MET-min·week^{-1}, total vigorous intensity activity MET-min·week^{-1} and total physical activity MET-min·week^{-1}. These data were calculated according to the IPAQ scoring system (International Physical Activity Questionnaire, 2005).

7.2.7 Digestive health questionnaire

The digestive health questionnaire from the Standard Shift Work Index (Barton et al., 1995) was completed by participants. This questionnaire comprised eight questions relating to the frequency (almost never, quite seldom, quite often, almost always) of appetite disruption, avoidance of certain foods to prevent stomach upsets, nauseous, heartburn, digestion difficulties, bloated stomach or flatulence, constipation or diarrhoea and abdominal pain. Moreover, the data from these questions were summed to provide a total digestive health score.

7.2.8 Food frequency questionnaire

Dietary assessment was sought via the European Prospective Investigation of Cancer in Norfolk food frequency questionnaire (Bingham et al., 2001). The questionnaire consisted of a list of 130 foods. Accompanying each food item is a multiple response grid in which participants indicated how frequently the item is consumed over the last year. Nine frequency categories were present, ranging from never to more than 6 times per day. The questionnaire provided post-intervention was modified to assess food intake over the past month rather than year. Data from the food frequency questionnaire, with the aid of food tables, were used to estimate energy and macronutrient intake. Unfortunately, the vast majority of shift-workers

either did not return the questionnaire, returned it late or failed to provide information for 10 or more food items (Bingham et al., 2001). Consequently, it was deemed inappropriate to analyse the food frequency questionnaires.

7.2.9 Statistical analysis

Baseline comparisons between study groups with respect to age, shift-work experience and number of children were analysed with independent samples *t*-test or Mann-Whitney tests if a Gaussian distribution was not observed. Chi-square tests were used to determine if baseline values between groups in regard to gender, education level and marital status were different. A covariance analysis was undertaken, with treatment group as a fixed factor and baseline measurement as a covariate in order to obtain adjusted group differences in regard to both continuous and ordinal outcome variables (Vickers and Altman, 2001). Sullivan and D'Agostino (2003) demonstrated that analysis of covariance can accommodate an ordinal outcome variable. The covariate-controlled analysis was performed in an Intention-to-treat manner (Hollis and Campbell, 1999). Multiple imputations were performed in order to account for missing datum bias. Ten imputations for each missing value were created, generating 10 imputed data sets. Each data set was analysed according to the abovementioned covariance analysis. Results from the 10 analysis were then pooled. Sensitivity analysis (data not shown) indicated no significant differences in effects based on another approach (i.e., last observation carried forward) to missing data. All statistical procedures were performed via SPSS for Windows, version 17. Statistical significance was set at $P<0.05$. Data are presented as mean±standard error.

7.3 RESULTS

Participant flow through the study is demonstrated in Figure 7.1. A total of 44 shift-workers entered the minimisation process. Data with respect to age, gender, shift-work experience, marital status and number of children did not differ significantly between the control and intervention group at baseline (Table 7.1). Participant attrition was moderate, with 68% retention at study week 13. Attrition was greater in the control group (48%) than the intervention group (14%).

Table 7.1. Baseline characteristics of participants.

	Total sample	Control	Motivational Interviewing	*P* for comparisons between groups
N	44	23	21	
Age (years)	45±9	46±7	45±10	0.749
Gender (m/f)	17/27	9/14	8/13	0.944
Education				
Secondary	22	12	10	0.915
College	17	8	9	
University	5	3	2	
SW experience (years)	17±10	17±11	18±10	0.758
Marital status				
Single	9	4	5	0.903
Co-inhabiting	6	3	3	
Married	29	16	13	
Number of children	1±1	1±1	1±1	0.683

With baseline covariate control, BMI was significantly smaller at study week 13 in the intervention group (29.49±0.18 $kg \cdot m^2$) than control group (30.06±0.22 $kg \cdot m^2$; P=0.043; Table 7.2). Over the course of the study, the intervention group lost 0.69 $kg \cdot m^2$, whereas the control group gained 0.62 $kg \cdot m^2$ (Table 7.2). At the end of the study, waist-to-hip ratio was similar in the intervention (0.88±0.01) and control group (0.87±0.01; P=0.528; Table 7.2).

At study week 13, total digestive health score was similar in the intervention (14.60±1.14 units) and control group (15.81±1.65 units; P=0.267; Table 7.2). No significant differences between experimental groups were present with respect to frequency of appetite disruption, avoidance of certain foods to prevent stomach upsets, nauseous, heartburn, digestion difficulties, bloated stomach or flatulence, constipation or diarrhoea, or abdominal pain (P>0.05; Table 7.2). However, the difference between the control and intervention trial in regard to frequency of heartburn and abdominal pain approached statistical significance (P=0.071 and 0.079 respectively). Specifically, frequency of heartburn and abdominal pain was 2.6±0.4 and 2.1±0.2 in the control group and 1.8±0.3 and 1.7±0.2 in the intervention group, respectively.

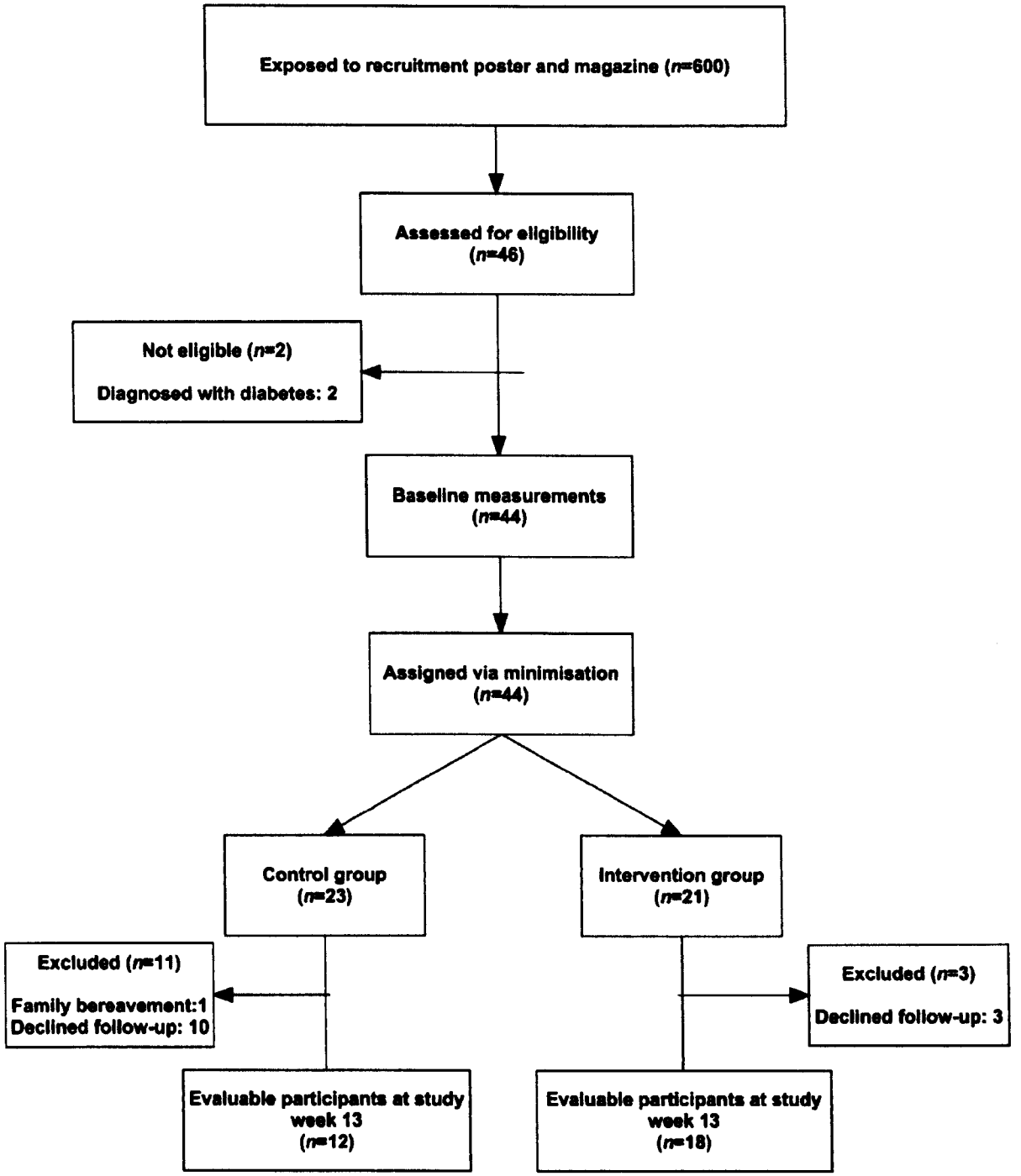

Figure 7.2. Study flow diagram.

Post-intervention total walking level was 645.93±232.26 MET-min·week^{-1} in the motivational interviewing group and 826.16±265.83 MET-min·week^{-1} in the control group (P=0.481; Table 7.2). Moderate intensity physical activity (e.g., brisk walking) level was similar at study week 13 in the intervention (4885.14±1461.30

MET-min·week^{-1}) and control group (3944.55±1781.56 MET-min·week^{-1}; *P*=0.349; Table 7.2). Post-study vigorous activity level was similar in the intervention (797.56±302.84 MET-min·week^{-1}) and control group (722.120±262.514 MET-min·week^{-1}; *P*=0.483; Table 7.2). At study week 13, total physical activity level was similar in the intervention (5832.97±1658.38 MET-min·week-1) and control group (4870.54±1272.56 MET-min·week-1; *P*=0.366).

Table 7.2. Baseline and post-study data for the control and intervention group.

	Baseline		Post-intervention		Effect
	Control group	Intervention group	Control group	Intervention group	
BMI (kg/m^2)	29.44±1.07	30.18±1.45	30.06±0.22	29.49±0.18	*P*=0.043
WHR	0.87±0.004	0.87±0.005	0.87±0.01	0.88±0.01	*P*=0.528
DH score	16.76±0.35	14.07±0.31	15.81±1.65	14.60±1.14	*P*=0.267
Disturbed appetite*	2.51±0.06	2.41±0.93	2.46±0.36	2.64±0.25	*P*=0.581
Stomach upset*	2.32±0.07	1.81±0.07	2.61±0.36	1.92±0.27	*P*=0.107
Nauseous*	1.77±0.06	1.50±0.05	1.80±0.27	1.51±0.19	*P*=0.309
Heartburn*	2.14±0.07	2.02±0.07	2.59±0.36	1.81±0.32	*P*=0.071
Digestive difficulties*	1.77±0.07	1.80±0.07	2.24±0.43	1.86±0.30	*P*=0.305
Bloated stomach*	2.23±0.07	2.42±0.05	2.12±0.27	1.83±0.17	*P*=0.340
Abdominal pain*	1.87±0.06	1.80±0.05	2.13±0.22	1.68±0.18	*P*=0.079
Constipation/ diarrhoea*	2.37±0.08	1.88±0.06	2.43±0.34	1.86±0.29	*P*=0.142
Walking	638.70±64.65	566.10±43.03	826.16±265.83	645.93±232.26	*P*=0.481
Moderate intensity PA	1798.83±182.04	1347.72±106.77	3944.55±1781.56	4885.14±1461.30	*P*=0.349
Vigorous intensity PA	765.82±68.16	947.33±84.14	722.120±262.514	797.56±302.84	*P*=0.483
Total PA	3256.06±227.51	2718.15±158.95	4870.54±1272.56	5832.97±1658.38	*P*=0.366

*BMI, body mass index; WHR, waist-to-hip ratio; DH, digestive health: *data refers to frequency of digestive symptom; all physical activity data are reported as MET-min·week⁻1.*

7.4 DISCUSSION

This is the first lifestyle intervention study specifically on UK-based shift-workers who do not require physical fitness for their job and remain awake during all their night-shifts. The intervention used in this study significantly reduced BMI, but not waist-to-hip ratio or the majority of digestive symptoms. The finding of a reduction in BMI after only 12-weeks of intervention in shift-workers is encouraging considering these

employees are at greater risk of having excess adiposity than day-workers (van Amelsvoort et al., 1999, Parkes, 2002, Suwazono et al., 2008). The positive finding with respect to BMI does not appear to relate to alterations in physical activity levels measured, but may be related to alterations in diet, although this study could not assess such a theory.

The finding of a significantly lower post-study BMI (or a greater reduction of BMI or body mass) of the shift-workers involved in the lifestyle motivational interviewing than control group supports some similar investigations (Carels et al., 2007, Elliot et al., 2007, Greaves et al., 2008, Hardcastle et al., 2008) but not all previous research (Resnicow et al., 2005, Befort et al., 2008, Webber et al., 2008). It is difficult to determine the exact reasons for the discrepancy between the above studies, but it is likely due to between-study differences in amount of motivational interviewing sessions and time given in each conference (Rubak et al., 2005), delivery method of motivational interviewing (face-to-face, telephone, internet) and time between baseline and post-study measurements. Interestingly, a study also involving shift-workers who did require physical fitness for their job and remained awake during all night-shifts reported no effect of supervised physical activity sessions upon BMI (Harma et al., 1988), indicating that motivational interviewing which focuses upon increasing physical activity level and improving dietary habits via individualised counselling could be a more effective strategy for reducing a shift-worker's BMI. Moreover, it is encouraging that motivational interviewing significantly reduced BMI in only 12 weeks in this study, considering its positive association with health problems prominent in shift-workers, such as cardiovascular disease (Field et al., 2001), cancer (Renehan et al., 2008) and hypertension (Dalton et al., 2003).

In this study, post-intervention waist-to-hip ratio was similar in the motivational interviewing and control group. One may assume that the significantly lower post-study BMI observed in the intervention than control group should have concurrently reduced waist-to-hip ratio. However, this did not occur, possibly because body mass was lost from areas other than the waist and hips such as the legs, arms or chest. Nevertheless, it is disappointing that waist-to-hip ratio was not affected by motivational interviewing considering its positive relationship with health problems prominent in shift-workers, such as cardiovascular disease (Rexrode et al., 1998, Hu et al., 2004), cancer (Giovannucci et al., 1995, Huang et al., 1999) and type 2 diabetes (Dalton et al., 2003).

In this investigation, post-study digestive health outcomes were not significantly different between the intervention and control group. These findings differ with the study by Harma et al. (1988) who reported a significantly greater post-study reduction in gastrointestinal symptoms in shift-workers randomised to the control group (-0.7%) rather than the physical activity intervention (0%). The aforementioned finding of Harma et al. (1988) is perplexing and should be viewed with caution due to 2 important limitations: (i) the authors did not use a covariance analysis with baseline measurement as a covariate in attempt to control for regression to the mean (Vickers and Altman, 2001) caused by baseline values being higher in the control than intervention group; (ii) an intention-to-treat analysis was not adopted and thus bias may have occurred (Hollis and Campbell, 1999). The digestive health findings from this study also contrast with those from a randomised controlled which demonstrated that increased physical activity level reduces the frequency of constipation in day-workers with irritable bowel syndrome (Daley et al., 2008). This discrepancy may be due to physical activity levels not being increased

significantly more in intervention than control trial in this study. Thus, a study which ensures physical activity levels are increased (e.g., via supervised exercise sessions) to a greater extent in shift-workers allocated to the intervention than control group is still needed to elucidate whether physical activity is related to digestive health in shift-workers.

In this study, no differences in regard physical activity levels were observed at follow-up which supports some similar studies (Elliot et al., 2007, Bennett et al., 2008) but not all (Harland et al., 1999, Brodie and Inoue, 2005, Bennett et al., 2007, Perry et al., 2007, Hardcastle et al., 2008). It is difficult to determine why some studies report no effect of motivational interviewing upon physical activity levels whereas others investigations observe an increase in response to the intervention. Between study differences, as highlighted above, relating to measurement of physical activity, baseline self-efficacy for exercise, frequency of motivational interviewing sessions and time given in each conference (Rubak et al., 2005), and time between baseline and post-study measurements may explain the discrepancy between the abovementioned studies in regard to physical activity. Surprisingly, in this study, post-intervention total physical activity level increased by 1614.48 MET-min·week^{-1} in the control group. This large increase at follow-up in total physical activity level may seem odd. However, both experimental groups received a healthy lifestyle booklet which contained information relating to the health benefits of increasing physical activity level and evidence exists which suggests that the screening process *per se* can increase an individual's physical activity level (Bankhead et al., 2003). Thus, the healthy lifestyle booklet alone or in combination with the screening process may explain why the control group increased their physical activity level over the study period.

This study has several limitations. First, it could be argued that insufficient diary information was obtained from participants and therefore it cannot be determined if the significantly lower post-study BMI of shift-workers in the intervention than control group was due to a reduction in energy intake for example. In hindsight, a primitive measure of dietary habits (e.g., fruit and vegetable intake) should have been administered post-study to explore if group differences were present. However, it should be noted the usefulness of data gained via a food frequency questionnaire is limited due to its poor validity compared to other dietary assessments such as weighed food diary (Cade et al., 2002). Second, attrition was significantly greater in the control than intervention group and thus a between group difference in the amount of missing data was present. Significant effort was expressed in an attempt to keep attrition to a minimal, but meeting testing schedules is fundamentally more difficult for the shift-worker compared to the day-worker for example. Future researchers should attempt to provide small incentives (e.g., free cinema or theatre tickets) for all participants to remain within the study. Third, the study sample size was sufficient to provide adequate statistical power for the primary outcome of BMI; however statistical power may have been insufficient in regard to secondary outcome variables (e.g., digestive health and physical activity level).

7.5 CONCLUSION

This study involved a unique population, UK shift-workers who do require physical fitness for their job and often undertake nocturnal wakefulness. In this population, a 12-week motivational interviewing intervention which focused upon increasing physical activity level and improving dietary habits significantly decreased BMI, but not waist-to-hip ratio or digestive symptoms. The positive finding in regard to BMI

was not due to increased energy expenditure via physical activity and therefore may be related to improved dietary habits (e.g., less energy intake). Long-term (i.e., over years) motivational interviewing focussing upon lifestyle factors may be a useful strategy for tackling excess adiposity in shift-workers.

CHAPTER 8

SYNTHESIS OF FINDINGS

8.1 INTRODUCTION

The purpose of this Chapter is to summarise and critically analyse the various findings from Chapters 3 to 7 in this thesis. In the first part of Chapter 8, the original objectives are returned to with respect to how they have been realised. The main findings from this thesis are then analysed collectively and an overall conclusion is presented. Finally, future research avenues resulting from the work presented in this thesis are outlined.

8.2 RELISATION OF OBJECTIVES

Objective 1: *Determine, using a cross-sectional study design, the general relationships between LTPA, body mass index and digestive symptoms in shift-workers.*

Objective 1 was achieved by conducting the first detailed cross-sectional survey solely involving shift-workers from different occupations and the findings from this assessment are presented in Chapter 3. The data from this study indicated that the least active shift-workers had the highest mean BMI. Nevertheless, dose-response effects of LTPA on BMI were not evident. A positive relationship was present between physical activity level and frequency of heartburn but not other digestive symptoms (e.g., constipation).

Objective 2: *Determine, via an experimental study design, the acute effects of evening exercise on appetite-related factors during a simulated night-shift whilst a 'typical' feeding schedule is used.*

This objective was achieved by conducting the first cross-over type nocturnal experiment on exercise and appetite-related factors, and the findings are presented in Chapter 4. Data from this study indicates that a prior bout of early evening exercise has no effect on average hunger but increases circulating levels of acylated ghrelin and leptin during a simulated night-shift compared with no prior exercise. The protocol in this experiment was characterised by a feeding schedule typically adopted by many shift-workers, i.e. eating smaller but more frequent portions of food rather than one large meal.

Objective 3: *Determine, via an experimental study design, the acute effects of evening exercise on appetite-related factors during a simulated night-shift whilst an 'atypical' feeding schedule is used.*

This objective was achieved by conducting a further cross-over type experiment and the findings are presented in Chapter 5. The data from this study indicates that a prior bout of early evening exercise has no effect on average hunger or plasma level of acylated ghrelin, but increases serum leptin concentration during a simulated night-shift compared with no prior exercise. The protocol in this experiment was characterised by a feeding schedule designed to be more in line with what day-workers do during their work-period, i.e. eating one larger meal 3-4 h after the work period has started.

Objective 4: *Determine, using appropriate within-subjects correlation analyses, the relationships between different factors that regulate appetite in the post-exercise period.*

This objective was achieved by pooling all the data from the exercise trials in chapters 4 and 5. A within-subjects covariate-controlled general linear model approach was adopted to describe a correlation matrix between the various factors. The findings are presented in Chapter 6. The data from this study indicates that exercise-related changes in plasma acylated ghrelin concentration are related to those in circulating levels of glucose and insulin, but not those in NEFA or triglyceride. Post-exercise changes in serum leptin level were also found to be related to those in circulating levels of insulin but not those in glucose, NEFA or triglyceride. The exercise-related changes in circulating level of acylated ghrelin, but not leptin were associated with the changes in hunger during the post-exercise period throughout the night.

Objective 5: *Determine, using a randomised controlled trial, the effect of altering (via motivational interviewing over a three-month period) a shift-worker's physical activity and dietary habits on their adiposity and digestive symptoms.*

This objective was achieved by conducting a randomised controlled trial and the findings are presented in Chapter 7. The data from this study indicates that a 12-week motivational interviewing intervention which focused upon increasing physical activity level and improving dietary habits significantly attenuated an increase in BMI, but not waist-to-hip ratio or frequency of digestive symptoms in UK shift-workers that remain awake during all night-shifts.

8.3 GENERAL DISCUSSION

The overall aim of this thesis was to determine the relationships between BMI, digestive health, appetite regulation and leisure-time physical activity during shift-work and night-work. Despite the fact that there 3.6 million shift-workers in the UK and these people are confronted by significant chronobiological and behavioural disturbances, there is a dearth of research on this topic. Data gained from studies involving shift-workers and simulated night-shifts also provide novel information regarding associations between physical activity and the abovementioned factors. Thus, this thesis makes a significant contribution to the field of physical activity and shift-work.

8.3.1 Chapter 3: Relationship between leisure-time physical activity, body mass index and digestive symptoms in shift-workers

Shift-work is a risk factor for excess adiposity and digestive problems (Atkinson et al., 2008). Prospective studies demonstrate that LTPA prevents excess body mass gain in day-workers (Owens et al., 1992, Williamson et al., 1993, Taylor et al., 1994, Haapanen et al., 1997). Moreover, some researchers have reported an inverse association between physical activity and frequency of some digestive symptoms (e.g., constipation) in day-workers (Daley et al., 2008). However, no data regarding the associations between LTPA, adiposity and frequency of digestive symptoms in shift-workers has been published. Thus, the relationships between LTPA, BMI and frequency of gastrointestinal problems were explored in shift-workers using a cross-sectional study design and the findings are presented in Chapter 3.

An inverse relationship between LTPA and BMI in shift-workers was observed, which is consistent with similar studies involving day-workers (Cooper et

al., 1976, Gibbons et al., 1983). However, a dose-response relationship between physical activity and BMI was not observed which contrasts with similar studies involving day-workers (Ross and Janssen, 2001). As discussed in Chapter 3, the lack of a dose-response relationship between LTPA and BMI is difficult to interpret and explain but may be due to measurement error. For example, LTPA levels, body mass and height were all self-reported and this probably introduced some error into the statistical model employed. Nevertheless, an inverse association was present. Despite this, the study provided no information regarding how (e.g., suppression of acylated ghrelin and thus hunger which in turn may decrease food intake or prevent compensatory increase in calories due to energy expended in response to exercise) or if physical activity prevents body mass gain in shift-workers.

A positive relationship between LTPA and frequency of heartburn in shift-workers was observed. Studies involving day-workers have shown that an acute bout of physical activity causes the heartburn, possibly by inducing gastro-oesophageal acid reflux (Clark et al., 1989). Thus, this may explain why a positive relationship between LTPA and frequency of heartburn was found. LTPA was not associated with any other digestive health problem measured. This is surprising considering that an inverse relationship between physical activity and some digestive health problems (e.g., constipation) has been observed in day-workers (Daley et al., 2008). However, the causes of digestive problems in shift-workers (e.g., disruption of circadian rhythms) and day-workers are probably different (Vener et al., 1989). Therefore this may explain why this study did not find an inverse relationship between LTPA and digestive problems such as constipation.

Three important questions emerged from Chapter 3. First, does an acute bout of physical activity have a favourable effect on regulators of hunger during a

subsequent night-shift? Second, does physical activity prevent excess adiposity in shift-workers? Third, does physical activity increase the frequency of heart burn in shift-workers? The answers to these questions were sought by undertaking the further studies, presented in Chapters 4, 5 and 7.

8.3.2 Chapter 4: Acute effects of evening exercise on appetite-related factors during a simulated night-shift whilst a 'typical' feeding schedule is used

Researchers have shown that an acute bout of short-term exercise suppresses plasma level of acylated ghrelin (Broom et al., 2007, 2009) and decreases (Elias et al., 2000, Legakis et al., 2004, Jurimae and Jurimae, 2005) or has no effect on serum leptin concentration (Kraemer et al., 1999a, Weltman et al., 2000, Fisher et al., 2001, Zafeiridis et al., 2003, Sari et al., 2007). These findings explain, in part, why an acute bout of exercise suppresses hunger (Thompson et al., 1988, King et al., 1994, King and Blundell, 1995, Westerterp-Plantenga et al., 1997, Broom et al., 2007, 2009) which in turn provides a reason why a compensatory increase in calories due to energy expended in response to physical activity does not occur (Jankowski and Foss, 1972, Thompson et al., 1988, Horton et al., 1994, King et al., 1994, 1996, 1997, Tremblay et al., 1994, Almeras et al., 1995, King and Blundell, 1995, Imbeault et al., 1997, Gilsenan et al., 1998, Lluch et al., 1998). Thus, the abovementioned responses to an acute bout of short-term exercise may explain why physical activity prevents body mass gain in individuals. However, all previous studies investigating the effect of exercise on appetite related factors have been undertaken during the day time and thus the findings may not be applicable to night-workers. Circadian variation exists in responses to exercise (e.g., see Kanaley et al., 2001) which indicates that the response of appetite-related factors to evening physical activity may differ from studies undertaken during the day time. Thus, an

experiment was undertaken to determine if exercise-mediated responses of hunger, plasma acylated ghrelin and serum leptin are different when awake and eating during a simulated night-shift compared with the findings from previous studies undertaken during the day. The findings from the study are presented in Chapter 4.

An early-evening bout of exercise mediated higher values of circulating concentrations of acylated ghrelin and leptin during a subsequent night-shift compared to no prior exercise. Moreover, early-evening exercise had no effect on mean hunger ratings during a subsequent night-shift. These findings contrast with those from the majority of similar investigations undertaken during the daytime involving measures of circulating levels of acylated ghrelin (Broom et al., 2007, 2009) and leptin (Kraemer et al., 1999a, Weltman et al., 2000, Fisher et al., 2001, Zafeiridis et al., 2003, Sari et al., 2007) and hunger ratings (Thompson et al., 1988, King et al., 1994, King and Blundell, 1995, Westerterp-Plantenga et al., 1997, Broom et al., 2007, 2009).

Insulin (Mohlig et al., 2002) and NEFA (Gormsen et al., 2006) are known to suppress circulating ghrelin level. Circulating levels of insulin and NEFA were found to be higher during the simulated night-shift when evening exercise had preceded it. These findings offer evidence that the relationships between ghrelin, insulin and NEFA change during a period of nocturnal wakefulness preceded by exercise.

Intravenous infusion of insulin or triglyceride increases circulating level of leptin. Circulating levels of insulin and triglyceride were higher during the simulated night-shift when evening exercise had preceded it. Thus, the post-exercise increases in circulating insulin and triglyceride levels may have concurrently increased serum leptin level.

The concurrent post-exercise responses of circulating levels of acylated ghrelin and leptin may have negated both hormones contrasting effects on appetite. Thus, this may explain why mean hunger during the simulated night-shift was unaffected by prior evening exercise.

It is difficult to explain why the abovementioned findings contrast with the majority of similar studies undertaken during the day time. Circulating levels of leptin increase in response to cortisol (Dagogo-Jack et al., 2005). Post-exercise serum cortisol response exhibits a circadian variation, with a larger response occurring in the night compared to morning (Kanaley et al., 2001). This time of day effect may explain why this investigation presented in Chapter 4 observed an increase in post-exercise serum leptin instead of no response communicated in several day time studies (Kraemer et al., 1999a, Weltman et al., 2000, Fisher et al., 2001, Zafeiridis et al., 2003, Sari et al., 2007).

8.3.3 Chapter 5: Acute effects of evening exercise on appetite-related factors during a simulated night-shift whilst an 'atypical' feeding schedule is used

In Chapter 4, a 'typical' feeding schedule (i.e., two small meals simulating grazing) was employed. However, it is acknowledged in some large organisations, adequate canteen facilities are available during the night which may allow the night-worker to have one large meal midway through the work period in the same way as a day-worker would have lunch rather than multiple smaller meals or snacks. The frequency of food intake has a significant effect on postprandial metabolic (e.g., NEFA) and hormonal (insulin and growth hormone) responses (Jenkins et al., 1990, 1992, Wolever, 1990, Bertelsen et al., 1993). Considering the aforementioned metabolites and hormones regulate circulating concentrations of ghrelin and leptin (Malmstrom et al., 1996, Mohlig et al., 2002, Freda et al., 2003, Gormsen et al.,

2006), it was hypothesised that the exercise-mediated response of these two regulators of appetite during nocturnal wakefulness would differ when consuming one large serving of food rather than two small meals during the night. Consequently, an experiment was undertaken to examine the effects of an acute bout of evening exercise upon circulating levels of acylated ghrelin and leptin during a simulated night-shift in which one large meal is consumed. The findings from the study are presented in Chapter 5.

The results from the study indicate that during a simulated night-shift in which one large test meal is consumed, prior evening exercise does not affect plasma acylated ghrelin concentration, but increases serum leptin level. In the previous study (presented in Chapter 4) involving ingestion of two smaller meals, circulating concentrations of both acylated ghrelin and leptin were increased by exercise. Altogether, these results indicate that the influence of exercise upon plasma acylated ghrelin concentration during a simulated night-shift is dependent on nocturnal meal frequency.

Mean postprandial circulating levels of some factors (e.g., insulin, NEFA and growth hormone) that suppress circulating levels of total ghrelin are significantly higher when meal frequency is low compared to high (e.g., two large meals vs. six smaller meals; (e.g., two large meals vs. six smaller meals; Jenkins et al., 1990, 1992, Wolever, 1990, Bertelsen et al., 1993). Thus, meal frequency may have an effect upon circulating levels of acylated ghrelin; however this theory has not been formally examined. The mean relative postprandial circulating concentrations of insulin (see Figure 8.1 for a comparison), NEFA and growth hormone could have been greater in this study (presented in Chapter 5) which employed two test meals rather than three in Chapter 4. Circulating concentrations of insulin, NEFA and

growth hormone are probably stronger regulators of plasma acylated ghrelin level than physical activity and this may explain why night-shift plasma acylated ghrelin level was unaltered by prior exercise in Chapter 5 unlike in Chapter 4.

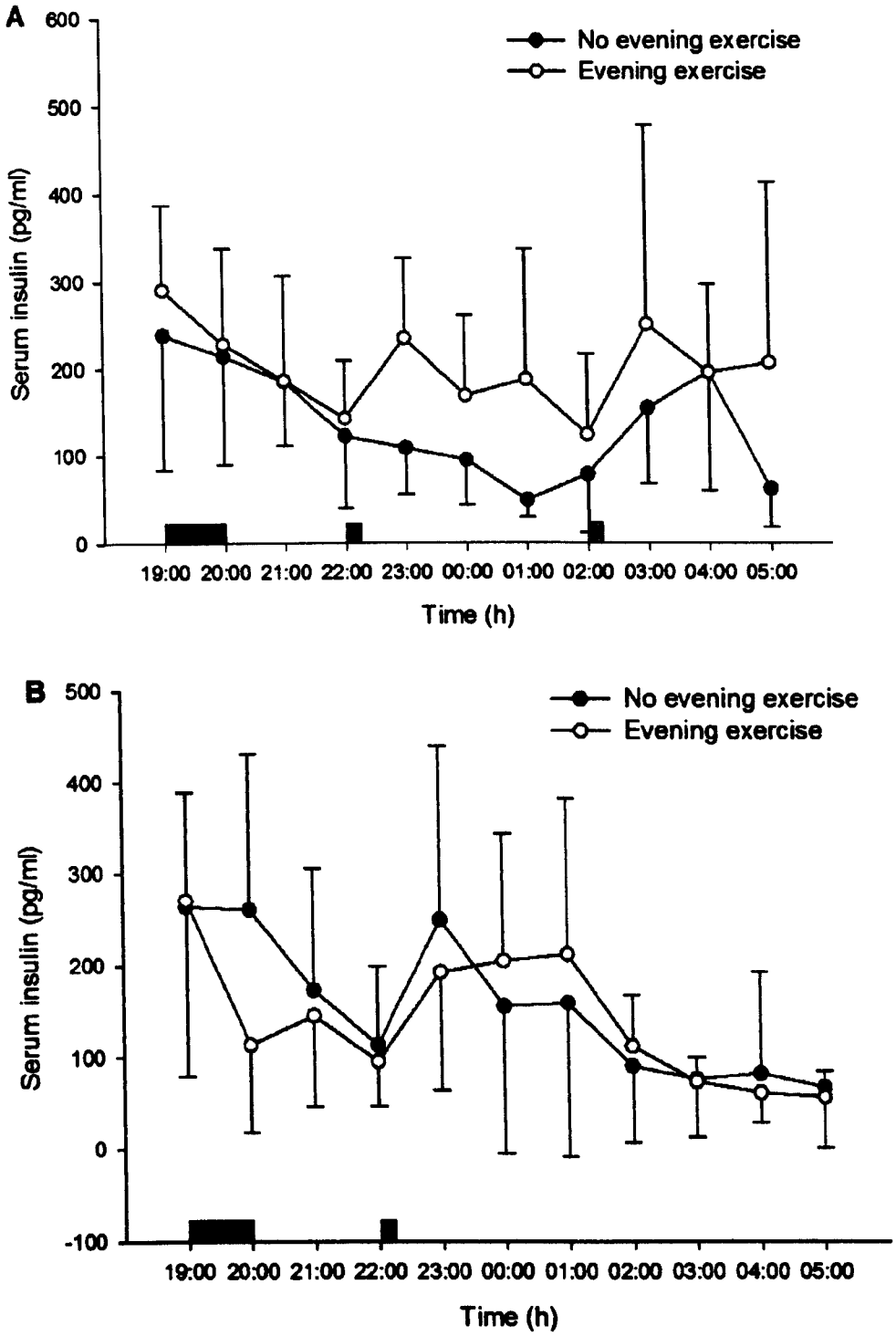

Figure 8.1. Comparison of serum insulin data reported in Chapters 4 (**A**) and 5 (**B**) (mean±SD). *Solid rectangle, cycling; grey square, test meal consumption.*

The effect of exercise upon serum leptin level during a simulated night-shift is not dependent on nocturnal meal frequency. This is difficult to explain considering a factor (i.e., insulin) which regulates leptin secretion is altered by meal frequency. Although speculative, it is possible that acylated ghrelin is more sensitive to changes in factors such as insulin than leptin. This may explain why the effect of exercise upon plasma acylated ghrelin level during a simulated night-shift is dependent on nocturnal meal frequency but serum leptin concentration is not.

Despite the abovementioned findings in regard to acylated ghrelin and leptin, hunger was unaffected during a night-shift preceded by exercise. Thus, findings taken together from Chapters 4 and 5 indicate that evening exercise does not affect hunger during subsequent night-shift. Consequently, a compensatory increase in calories due to energy expended in response to exercise may not occur, inducing a negative energy balance which might cause body mass loss overtime. Furthermore, this may explain why an inverse association between LTPA and BMI was found in Chapter 3.

8.3.4 Chapter 6: Within-subject relationships between post-exercise concentrations of acylated ghrelin, leptin and other variables related to human metabolism and hunger

Factors (i.e., hormones and metabolites) relating to circulating levels of ghrelin and leptin have been explored at rest (e.g., Gormsen et al., 2006, Laferrere et al., 2006). In comparison, relatively little research has been undertaken to determine if some commonly researched variables (e.g., NEFA and triglyceride) relate to circulating levels of acylated ghrelin and leptin post-exercise. Consequently, post-exercise (20:00 to 22:00 h) data from Chapters 4 and 5 were combined to determine if hunger and circulating levels of insulin, glucose, NEFA and triglyceride relate to circulating

concentrations of acylated ghrelin and leptin. The findings from this study are presented in Chapter 6.

Post-exercise circulating concentrations of acylated ghrelin were inversely correlated with plasma glucose concentration. Moreover, an inverse association between circulating levels of acylated ghrelin and insulin approached statistical significance (P=0.052). These two findings confirm that acylated ghrelin is related to glucose and insulin post-exercise like at rest. However, there is no indication of causality and it is possible that ghrelin controls glucose and insulin or vice versa after exercise as observed in studies involving individuals at rest (Broglio et al., 2001, 2003, Mohlig et al., 2002, Kim et al., 2007). Broom et al. (2007, 2009) reported no relationship between circulating levels of glucose, insulin and acylated ghrelin which differs with the findings presented in Chapter 6. Between study differences in regard to exercise intensity utilised and time of day (early morning vs. evening) that exercise occurred may explain the conflicting findings.

There was no evidence of a correlation between circulating acylated ghrelin level and plasma concentrations of NEFA or triglyceride. Thus, this indicates that the relationship observed between ghrelin, NEFA and triglyceride at rest (Gormsen et al., 2006, Banks et al., 2008) is abolished post-exercise.

A positive association between post-exercise plasma concentrations of acylated ghrelin and hunger was present in Chapter 6. This is an important finding and supports previous studies (Broom et al., 2007, 2009), it suggests that the association between hunger and acylated ghrelin is present after exercise as well at rest (Wren et al., 2001a). Thus, this indicates that post-exercise levels of acylated ghrelin are in important in regard to energy balance regulation.

A positive association between post-exercise circulating levels of leptin and insulin was reported in Chapter 6. However, of course there is no indication of causality. This matter, in a comparable manner to that discussed above regarding acylated ghrelin, glucose and insulin, is complicated by experimental studies indicating that leptin increases (Ahren and Havel, 1999) and is suppressed by insulin (Malmstrom et al., 1996, Utriainen et al., 1996). Thus, one cannot be certain in regard to causality between post-exercise levels of leptin and insulin.

Neither post-exercise levels of glucose, NEFA or triglycerides correlated with serum level of leptin in Chapter 6. Thus, it appears that the regulatory effects of glucose (Levy and Stevens, 2001), NEFA (Evans et al., 2001, Stefan et al., 2001, Garcia-Lorda et al., 2003) and triglycerides (Banks et al., 2004) on circulating leptin level at rest are eradicated after exercise.

In Chapter 6, no association between serum leptin level and hunger was present post-exercise. This finding is interesting because if the exercise-mediated decrease in circulating leptin level occurs, as reported by some researchers (Elias et al., 2000, Legakis et al., 2004, Jurimae and Jurimae, 2005) but not all (Weltman et al., 2000, e.g., Fisher et al., 2001, Sari et al., 2007), it is unlikely to stimulate post-exercise hunger and thus energy consumption.

8.3.5 Chapter 7: Effects of a lifestyle intervention based on motivational interviewing on shift-worker's adiposity and digestive health: a randomised controlled trial

Findings from Chapter 3 indicate that LTPA is related to BMI and heartburn in shift-workers. Moreover, the results from Chapters 4 and 5 suggest night-shift hunger is not affected by prior evening exercise. Therefore, a compensatory increase in energy consumption due to the energy expended in response to exercise may not

arise and thus a negative energy balance would occur which might induce body mass loss overtime. However, a long-term randomised controlled trial is required to determine decisively if physical activity influences shift-worker's adiposity and digestive health.

Only two randomised controlled trials have investigated the effect of a lifestyle intervention upon shift-worker's digestive health and/or adiposity and they have produced conflicting findings (Harma et al., 1988, Elliot et al., 2007). In the study conducted by Elliot et al. (2007), the lifestyle intervention was delivered to US fire-fighters via motivational interviewing. However, fire-fighters are generally permitted to sleep during the night-shift whilst they are not attending an emergency. Therefore, the adverse effects of nocturnal wakefulness might not apply as readily to fire-fighters as to police communications officers for example. Consequently, a randomised controlled trial was undertaken to determine conclusively if an individualised lifestyle (physical activity and diet) intervention is influential in regard to the adiposity and digestive health of relatively physically inactive (compared to fire-fighters for example; see Fullick et al., 2009) UK shift-worker's involved in police communications. The findings from this study are presented in Chapter 7.

The lifestyle intervention significantly reduced the shift-worker's BMI, but not waist-to-hip ratio or frequency of digestive symptoms. There was no difference between the control and intervention group in physical activity level. This indicates that a reduction in energy intake, rather than energy expenditure was the reason why the intervention, rather than control group, lost more body mass over time. However, due to a methodological problem (i.e., a sufficient quantity of food frequency questionnaires were not returned) no data were available to support or refute this postulation.

Both the control and intervention group increased their physical activity levels over time. Thus, the findings from Chapter 7 cannot shed light on the relationship between LTPA, BMI and heartburn reported in Chapter 3. Therefore, it is still unclear if physical inactivity causes excess adiposity and/or heartburn, or whether physical inactivity is a consequence of excess adiposity and/or heartburn in shift-workers.

8.3.6 Conclusion

Relationships exist between LTPA, BMI and one digestive symptom (i.e., heartburn) in shift-workers. However, more experimental research is required to determine if a shift-worker's BMI and feeling of heartburn is a consequence or cause of physical inactivity.

A bout of evening exercise increases circulating concentrations of factors (i.e., acylated ghrelin and leptin) that regulate hunger during a simulated night-shift when a 'typical' feeding schedule is undertaken. However, during a simulated night-shift in which an 'atypical' feeding schedule is utilised, plasma acylated ghrelin level is unaffected, but serum leptin concentration is increased by a bout of prior evening exercise. Taken together, the abovementioned findings indicate that meal frequency is an important factor which regulates the response of acylated ghrelin during a simulated night-shift preceded by evening exercise. Despite the above findings, mean night-shift hunger was unaffected by evening exercise, regardless of meal frequency. This suggests a compensatory increase in calories due to energy expended in response to exercise may not occur, inducing a negative energy balance which might cause body mass loss in shift-workers.

An individualised lifestyle intervention delivered via motivational interviewing significantly reduces BMI, but has no effect waist-to-hip ratio or frequency of

digestive problems in shift-workers overtime. The reduction in BMI was not due to an increase in energy expended via physical activity and thus was presumably a result of decreased food intake.

8.3.7 Future directions

1. A comparison of physical activity level, measured objectively (e.g., via actimetry), between day- and shift-workers needs to be undertaken. This will determine if physical activity differs between day- and shift-workers. Such data will help explain if the adverse effect of shift-work on health (e.g., excess adiposity) is mediated by physical inactivity or not.

2. The effect of evening exercise on night-shift responses of other factors (e.g., pancreatic polypeptide, peptide YY, cholecystokinin, oxyntomodulin and cortisol) that regulate food intake needs to be assessed. This will provide a more comprehensive understanding of the effect of evening exercise upon night-shift responses of factors that regulate appetite.

3. The effect of evening exercise intensity on night-shift responses of factors that regulates energy intake needs to be investigated. This will determine if specific exercise intensities are favourable or harmful in regard to night-shift appetite regulation.

4. The effect of evening exercise on *ad labitum* food intake during a night-shift needs to be investigated. This will determine if a compensatory increase in calories due to energy expended in response to exercise occurs or not. Such

5. The long-term (>1 year) effect of physical activity *per se* on adiposity and digestive health of shift-workers needs to be addressed. Such a study will determine if physical activity *per se* should be prescribed to shift-workers in order to prevent excess adiposity and reduce the frequency of digestive symptoms.

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