The effects of accumulating physical activity on postprandial lipaemia and other cardiovascular disease risk factors

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THE EFFECTS OF ACCUMULATING PHYSICAL ACTIVITY ON POSTPRANDIAL LIPAEMIA AND OTHER CARDIOVASCULAR DISEASE RISK FACTORS

by

Masashi Miyashita

A Doctoral Thesis

Submitted in partial fulfillment of the requirements for the award of Doctor of Philosophy of Loughborough University

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ABSTRACT

Cardiovascular disease is a major cause of death in many developed countries. Impaired clearance of postprandial triacylglycerol, small increases in blood pressure and elevated C-reactive protein concentrations (a marker for inflammation) have been associated with an increase in cardiovascular disease risk. To reduce such risk factors, the unique concept of accumulating physical activity was introduced to the general public, advising that activity can be performed in several short bouts throughout the day with a minimum duration of 10 minutes per activity bout. Although there is evidence to support the benefits of accumulating exercise on health, little is known regarding the health effects of accumulating exercise in bouts lasting less than 10 minutes.

The broad theme of this thesis is therefore to investigate the effects of accumulating short (<10 minute) bouts of exercise throughout the day on postprandial lipaemia, an emerging risk marker for cardiovascular disease, and other cardiovascular disease risk markers including resting blood pressure and C-reactive protein. In addition, acylated ghrelin (a hormone involved in energy balance) was examined in one of the studies reported here since this is a topical research area in energy metabolism which relates to appetite regulation. This has relevance for weight control which in turn has an influence on cardiovascular disease risk.

The first study examined the effect of accumulating a large volume (4.18 MJ = 1000 kcal) of exercise in short (6 minute) bouts performed throughout the day on postprandial triacylglycerol concentrations and resting blood pressure in young healthy men. Accumulating short bouts of running was effective in lowering postprandial triacylglycerol concentrations and resting systolic blood pressure on the following day.

The second study extended the findings of the first study by demonstrating that a smaller volume of exercise (1.96 MJ = 476 kcal) (30 minute) accumulated in very short (3 minute) bouts of running is equally effective in reducing postprandial triacylglycerol concentrations on the following day as one continuous 30 minute run in healthy young men. In addition, accumulating 30 minutes running throughout the day was as effective as one continuous 30 minute run in reducing resting systolic blood pressure on the following day in normotensive men.
The third study reported here extends the findings of the previous two studies by examining short bouts of walking as opposed to running exercise. Accumulating ten, very short (3 minute) bouts of self-selected brisk walking (1.10 MJ = 265 kcal) over the course of one day reduced postprandial triacylglycerol concentrations on the following day. The magnitude of this attenuation was similar to that observed when activity was performed in a single session of 30 minutes continuous brisk walking. In addition, both patterns of walking lowered resting systolic blood pressure measured on the following day in normotensive men.

The final study reported here examined whether accumulating short bouts of running, performed on the same day as test meals are consumed, lowers postprandial triacylglycerol concentrations in healthy young men. In addition, serum high-sensitivity C-reactive protein and plasma acylated ghrelin were measured to investigate whether these factors are affected by accumulated activity. Accumulating short (5 minute) bouts of running throughout the day (total of 30 minutes: 1.76 MJ = 420 kcal) reduced postprandial triacylglycerol concentrations. There were no differences in serum high-sensitivity C-reactive protein and plasma acylated ghrelin concentrations between the trials and over time.

In conclusion, these data indicate that health benefits can accrue, at least in terms of lowering postprandial triacylglycerol concentrations and resting blood pressure, through the accumulation of short (<10 minute) bouts of exercise. These findings suggest that accumulating short bouts of exercise may offer some protection from cardiovascular disease.
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This thesis is dedicated to
my wife Saori, my parents, two sisters, and my parents-in-law.
PREFACE

Some of the findings presented in this thesis has been published as follows:

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CHAPTER 1
INTRODUCTION

In the United Kingdom, recent statistics reveal that there are approximately 233,000 deaths from coronary heart disease per year (Petersen et al., 2004). The main cause of death comes from cardiovascular disease of which coronary heart disease and stroke are the two main forms. The World Health Organisation reports that physical inactivity is a major risk factor for cardiovascular disease (World Health Organization, 2002). Although there are numerous sources of evidence that regular physical activity is beneficial for health (Kesaniemi et al., 2001), only 31% of adults are active (U.K. Department of Health, 2004) (active is defined as achieving at least 30 minutes of moderate intensity physical activity on 5 or more days of the week). Therefore, in an effort to reduce the prevalence of lifestyle related diseases, several governmental bodies and authorities have begun to promote physical activity as a valuable therapeutic tool.

In 1995 the Centers for Disease Control and Prevention and the American College of Sports Medicine published physical activity guidelines which stated that adults should accumulate 30 minutes of moderate intensity activity on most, preferably all, days of the week (Pate et al., 1995). The concept of accumulating physical activity was novel at this time, but the guidelines included the caveat that the minimum duration of any one bout should be 10 minutes. The US National Institutes of Health (U.S. Department of Health and Human Services, 1995) and the US Surgeon General’s Report (U.S. Department of Health and Human Services, 1996) reported similar recommendations in the same year and the following year, respectively. Moreover, the concept of
accumulation has been endorsed recently by the Department of Health in the United Kingdom (2004). Again a minimum exercise duration of 10 minutes is recommended for individual activity bouts.

The rationale behind the concept of accumulation for health is based on the assumption that the activities reported in many epidemiological studies are intermittent in nature (e.g. walking, stair climbing, gardening, occupational work) (Morris et al., 1953; Morris and Crawford, 1958; Paffenbarger et al., 1978; Paffenbarger et al., 1986). The nature of many of these activities is such that activity may have been accumulated throughout the day rather than being performed in one continuous bout. However, there is insufficient scientific evidence to support the notion of accumulation for health although at least two intervention studies (DeBusk et al., 1990; Ebisu, 1985) were available at the time of the first guideline (Pate et al., 1995) and they were cited at the time. However, recently the findings of at least one prospective study indicate that accumulating short bouts of physical activity confers equal benefit in reducing coronary heart disease risk as performing one longer bout, providing that the total amount of energy expended is similar (Lee et al., 2000).

Several intervention studies have investigated the issue of accumulating exercise for health. The majority of these studies have reported that regular accumulated exercise confers a positive benefit on fitness, blood lipids, blood pressure, blood glucose, insulin and body composition (Table 2.1). However, existing intervention studies need to be interpreted with caution. Most of the intervention studies concerning on accumulated exercise have used self-reported diaries to quantify physical activity and several studies have not included a control group. Hence, randomised, controlled
intervention studies are needed to support recent physical activity guidelines for health (U.K. Department of Health, 2004; Pate et al., 1995; U.S. Department of Health and Human Services, 1996).

The majority of accumulation studies have employed activity bouts with a minimum duration of 10 minute per bout. This is not surprising because this is consistent with the physical activity recommendations stated earlier. However, if total energy expenditure is the most important factor for health benefits, as is suspected (Pate et al., 1995), then the duration of exercise should not matter provided that sufficient energy is expended. This is an important point, because activities that are intermittent in nature often involve bouts lasting less than 10 minutes (i.e. walking, stair climbing, golfing, housework, gardening and carpentry amongst others). Moreover, even amongst people who follow the recommended physical activity guidelines of 30 minutes on most if not all days of the week (Pate et al., 1995) most people do not exercise continuously for 10 minutes at a time (Whitt et al., 2003).

With regard to cardiovascular disease, a delayed clearance of postprandial triacylglycerol and high blood pressure are considered cardiovascular disease risk factors (Stanner, 2005). With respect to the former, a previous case-control study has shown that a high postprandial concentration of triacylglycerol is a strong and independent risk predictor for cardiovascular disease (Patsch et al., 1992). With respect to the latter, it has been suggested that a small increase in blood pressure in healthy, normotensive men and women is linked to the risk of developing hypertension (Blair et al., 1984). With this in mind a recent report has introduced a new classification, the term 'prehypertension' (a systolic blood pressure ranging from 120-139 mm Hg and a
diastolic blood pressure ranging from 80-89 mm Hg) that requires early adoption of lifestyle modifications to prevent further increases in blood pressure and hence cardiovascular disease risk (Chobanian et al., 2003). An investigation for the effect of exercise on blood pressure in normotensive populations is therefore as clinically important as it is in hypertensive individuals.

With regard to postprandial lipaemia, atherosclerosis is now considered an inflammatory condition (Peter et al., 2002), and greater research attention has been given to acute- and chronic-phase inflammatory factors. It has been reported that increased C-reactive protein, a marker of inflammation, is associated with increased risk of cardiovascular disease in healthy men and women (Koening et al., 1999; Ridker et al., 2002). A previous study has shown that chronic exercise training reduces C-reactive protein concentration (Mattusch et al., 2000), suggesting that exercise might have a protective effect on inflammation possibly due to an enhancement of endothelium function (Walther et al., 2004).

In addition to postprandial triacylglycerol concentration, blood pressure and C-reactive protein, the gastrointestinal hormone, ghrelin provides another interesting area of study. This hormone is involved in the short-term regulation of food intake and energy homeostasis (Ariyasu et al., 2001). Regular exercise has a positive impact on the creation of negative energy balance. Since ghrelin concentration is related to energy balance (Cummings et al., 2001), exercise may modulate ghrelin concentrations due in part to resulting the energy deficit. It is therefore of interest to explore the combined influence of exercise and nutrient intake on plasma ghrelin concentration since this has implications for weight control and hence cardiovascular disease risk.
There is consistent evidence that postprandial triacylglycerol concentrations are reduced through a single bout of aerobic exercise (Gill and Hardman, 2003; Petitt and Cureton, 2003). Similarly, previous studies have shown that a single session of aerobic exercise reduces resting blood pressure in normotensive (Lockwood et al., 2005) and hypertensive (Brandao Rondon et al., 2002) individuals. A small number of studies have investigated the effect of accumulating exercise on postprandial triacylglycerol concentrations (Altena et al., 2004; Gill et al., 1998; Murphy et al., 2000), resting blood pressure (Hagberg et al., 1987; Kaufman et al., 1987) and ambulatory blood pressure (Padilla et al., 2005). Although these studies have provided support for the concept of accumulation, the acute effects of accumulating exercise in bouts shorter than 10 minutes per session throughout the day on these risk factors has not been determined. In addition, no study is available regarding the effect of accumulated exercise on C-reactive protein concentrations. Furthermore, although several studies have investigated the effect of an acute bout of exercise on total ghrelin concentrations (active and inactive ghrelin combined) (Dall et al., 2002; Kallio et al., 2001; Kraemer et al., 2004a; Schmidt et al., 2004; Zoladz et al., 2005), no study has investigated the effects of accumulated exercise on active ghrelin concentrations (active ghrelin is the biologically active form of the hormone). The study of many short bouts of exercise may be more representative of everyday life. Moreover, partitioning exercise into short bouts of exercise throughout the day may be applicable to sedentary populations who have low physical fitness.

Although the concept of accumulating physical activity has been widely publicised and intervention studies are available (Table 2.1), more controlled laboratory-based interventions are necessary to assess the effects of accumulating short bouts of exercise
on various aspects of health. In addition, there are few studies regarding the effect of accumulating short bouts of exercise with a duration lasting less than 10 minutes per bout on health. The main aim of this thesis is therefore to investigate the effect of accumulating short (<10 minute) bouts of exercise throughout the day on postprandial triacylglycerol concentrations. Additionally this thesis investigates the effects of accumulating short bouts of exercise throughout the day on other cardiovascular disease risk factors namely resting blood pressure and C-reactive protein. Acylated (active) ghrelin concentrations are also examined during exercise as a potential obesity-related hormone modulator. The findings from this thesis should provide support for recent public health guidelines on physical activity and provide additional information regarding the mechanisms by which physical activity can reduce cardiovascular disease risk.
CHAPTER 2
REVIEW OF LITERATURE

2.1 Introduction
The following review will address the available literature that has examined accumulated exercise and cardiovascular disease risk factors. Firstly, brief coverage of epidemiological evidence regarding exercise and health with emphasis on the role of accumulated exercise is mentioned. Thereafter, laboratory-based intervention studies that examine the acute and chronic effects of accumulating exercise on health-related outcomes, including aerobic fitness, lipid/lipoproteins, glucose, insulin and body composition are described. Thereafter, existing intervention studies that examine the effects of accumulating exercise on postprandial triacylglycerol concentrations and blood pressure are discussed. Literature concerning the effects of exercise on C-reactive protein and ghrelin concentrations are also briefly discussed at the end of this review.

2.2 Accumulating physical activity for health – epidemiological evidence
The weight of evidence suggests that exercise can play a pivotal role in reducing coronary heart disease risk. Jeremy Morris was the first researcher who systematically investigated the relationship between daily activity and the incidence of coronary heart disease. Morris et al (Morris et al., 1953; Morris and Crawford, 1958; Morris et al., 1980; Morris et al., 1990) demonstrated that higher occupational work or leisure-time physical activity was related to lower coronary heart disease incidence rates. These observations were supported and extended by large epidemiological studies, conducted by Paffenbarger et al (Paffenbarger et al., 1978; Paffenbarger et al., 1984; Paffenbarger
et al., 1986; Paffenbarger et al., 1993). The types of activities in these studies included walking, stair climbing, golf, housework, gardening and carpentry amongst others. The nature of many of these activities is such that activity may have been accumulated throughout the day rather than being performed in one continuous bout. In addition, no causal relationship has been clearly established to show that exercise is inversely related to coronary heart disease risk since epidemiological data only demonstrate associations.

To date, only one prospective study provides direct evidence that accumulating exercise is beneficial to health. In the Harvard Alumni Health Study, Lee et al (2000) conducted surveys of physical activity and medical history from 1989 to 1993 in 7307 middle-aged and elderly male Harvard alumni. The types of activities reported included walking, stair climbing, and sports or recreational activities such as golfing, gardening and skiing. The study examined the average time spent on each activity and estimated the total energy expenditure in physical activity. Health outcomes were assessed in these male alumni based on the incidence of coronary heart disease. The study found that sports and recreational activities are associated with decreased risk of coronary heart disease. Another interesting finding in this study was that after adjusting for the total energy expenditure of these activities, longer durations spent in each activity bout did not provide greater benefits in decreasing coronary heart disease risk compared with shorter durations. This finding suggests that accumulating short bouts of exercise has an equal benefit in reducing coronary heart disease risk as longer bouts of exercise when the same total energy is expended. However, caution is required when interpreting the results of this study. The activity levels in this report are based solely on observation, therefore the precise activity patterns required to protect from
coronary heart disease are unknown.

2.3 Accumulating physical activity for health – intervention studies

The effectiveness of accumulating short bouts of exercise to modify disease risk factors has been examined in many intervention studies (Table 2.1). The purpose of these studies was perhaps not only to strengthen on public health recommendations for physical activity, but also to provide practical guidance to the general public since participation in accumulated activity may be easier to incorporate into a daily schedule (DeBusk et al., 1990), particularly in sedentary populations (Jakicic et al., 1995). Most of these intervention studies have employed a multi-factorial approach, measuring several health-related outcomes concurrently (Table 2.1).

Table 2.1 Summary of intervention studies examining the effects of accumulated/intermittent exercise on health

<table>
<thead>
<tr>
<th>(Reference)</th>
<th>Study design</th>
<th>Subjects</th>
<th>Main findings on ACC exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Ebisu, 1985)</td>
<td>10 wk; running</td>
<td>M, young</td>
<td>Increased maximal oxygen uptake and HDL-C</td>
</tr>
<tr>
<td>(DeBusk et al., 1990)</td>
<td>8 wk; running</td>
<td>F&amp;M, middle-age</td>
<td>Increased maximal oxygen uptake and decreased body weight</td>
</tr>
<tr>
<td>(Jakicic et al., 1995)</td>
<td>20 wk; walking</td>
<td>F, young</td>
<td>Reduced oxygen uptake at HRpeak, decreased SBP, DBP and body weight</td>
</tr>
<tr>
<td>(Dunn et al., 1997)</td>
<td>6 mo; LPA</td>
<td>F&amp;M, middle-age</td>
<td>Increased maximal oxygen uptake and decreased TC, TC:HDL-C, SBP, DBP and % body fat</td>
</tr>
<tr>
<td>Study Authors</td>
<td>Duration</td>
<td>Type of Activity</td>
<td>Gender</td>
</tr>
<tr>
<td>-------------------------------</td>
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</tr>
<tr>
<td>Snyder et al., 1997</td>
<td>32 wk; walking</td>
<td>F, middle-age</td>
<td>No effects on maximal oxygen uptake, insulin and glucose levels</td>
</tr>
<tr>
<td>Murphy and Hardman, 1998</td>
<td>10 wk; walking</td>
<td>F, middle-age</td>
<td>Increased maximal oxygen uptake, decreased lactate thresholds, % body fat, body weight and waist circumference</td>
</tr>
<tr>
<td>Woolf-May et al., 1998</td>
<td>18 wk; walking</td>
<td>F&amp;M, middle-age</td>
<td>Decreased peak HR</td>
</tr>
<tr>
<td>Andersen et al., 1999</td>
<td>68 wk; LPA</td>
<td>F, middle-age</td>
<td>Increased maximal oxygen uptake and decreased TC, LDL-C, HDL-C and SBP</td>
</tr>
<tr>
<td>Clapp et al., 1999</td>
<td>Acute; walking</td>
<td>F&amp;M, middle-age with CFS</td>
<td>No abnormal cardiorespiratory responses to exercise</td>
</tr>
<tr>
<td>Coleman et al., 1999</td>
<td>32 wk; walking</td>
<td>F&amp;M, middle-age</td>
<td>Increased maximal oxygen uptake, and decreased DBP and % body fat</td>
</tr>
<tr>
<td>Dunn et al., 1999</td>
<td>24 mo; LPA</td>
<td>F&amp;M, middle-age</td>
<td>Increased maximal oxygen uptake and reduced submaximal HR, and deceased TC, SBP, DBP and % body fat</td>
</tr>
<tr>
<td>Jakicic et al., 1999</td>
<td>18 mo; walking</td>
<td>F, middle-age</td>
<td>Increased peak oxygen uptake and decreased BMI</td>
</tr>
<tr>
<td>Woolf-May et al., 1999</td>
<td>18 wk; walking</td>
<td>F&amp;M, middle-age</td>
<td>Reduced lactate and HR at the final stage of submaximal, decreased LDL-C</td>
</tr>
<tr>
<td>Boreham et al., 2000</td>
<td>7 wk; stair-climbing</td>
<td>F, young</td>
<td>Reduced lactate, TC and TC:HDL-C, and increased HDL-C</td>
</tr>
<tr>
<td>Donnelly et al., 2000</td>
<td>18 wk; walking</td>
<td>F, middle-age</td>
<td>Increased maximal oxygen uptake, decreased SBP, insulin, and increased HDL-C</td>
</tr>
<tr>
<td>Moreau et al., 2001</td>
<td>24 wk; walking</td>
<td>F, middle-age</td>
<td>Decreased body weight and SBP</td>
</tr>
</tbody>
</table>
(Schmidt et al., 2001) 12 wk; arm+leg cycling  F, young Increased maximal oxygen uptake and decreased body weight, BMI and % body fat

(Staffileno et al., 2001) 8 wk; walking/cycling  F, older Decreased SBP and DBP

(Asikainen et al., 2002) 15 wk; walking  F, older Increased maximal oxygen uptake and decreased body weight, BMI and % body fat

(Murphy et al., 2002) 6 wk; walking  F, middle-age Increased maximal oxygen uptake, increased HDL-C, and decreased TAG, TC and DBP

(Schachter et al., 2003) 16 wk; rhythmic movement (home-videotape-based)  F, middle-age No effects on maximal oxygen uptake or the duration of the exercise test

(Swartz et al., 2003) 8 wk; walking  F, middle-age Improved glucose tolerance and decreased SBP and DBP

(Sykes et al., 2003) 8 wk; walking/cycling  F, middle-age Decreased body weight, BMI, waist circumference, waist to hip ratio and % body fat

(Murtagh et al., 2004) 12 wk; walking  F, middle-age Reduced HR and RPE during the submaximal test

(Baynard et al., 2005) Acute; walking  F, middle-age with diabetes No effects on metabolic function (Oral glucose tolerance test)

(Boreham et al., 2005) Acute; stair-climbing  F, young Increased maximal oxygen uptake and decreased LDL-C

(Osei-Tutu and Campagna, 2005) 8 wk; walking  F&M, middle-age Increased maximal oxygen uptake

(Padilla et al., 2005) Acute; LPA  F&F, middle-age and older Decreased SBP (in prehypertensive and hypertensive groups)
Several studies have shown that there is an improvement in maximum oxygen uptake or predicted maximum oxygen uptake with accumulated exercise training (Murphy et al., 2002; Jakicic et al., 1995; Coleman et al., 1999; Donnelly et al., 2000). For example, DeBusk et al. (1990) examined the effect of different patterns of jogging on aerobic fitness in 18 healthy middle-aged men. Exercise regimes involved either three 10-minute bouts of exercise performed throughout the day or one 30-minute bout of continuous exercise, for 5 days a week over a period of 8 weeks. This study found a significant increase in maximum oxygen uptake in both groups at the end of training period (3 x 10 minutes: from 33 ± 3.2 to 37.9 ± 3.5 mL/kg/min; 1 x 30 minutes: from 32.1 ± 4.6 to 34.5 ± 4.5 mL/kg/min). In a similar exercise design, Murphy and Hardman (1998) demonstrated aerobic fitness improvement in 47 sedentary middle-aged women after a training programme involving accumulated activity. However, no improvements in maximum oxygen uptake were observed in young individuals who underwent either an accumulated exercise or a continuous exercise training programme (in both groups intensity was set at 50-60% of estimated maximum heart rate, and exercise performed 3 days a week for 12 weeks) (Thomas et al., 2001). The reason for the conflicting results observed between the studies of
Murphy and Hardman (1998) and Thomas et al (2001) is unclear. However, it is possible that the low pre-training maximum oxygen uptake values in the study of Murphy and Hardman (1998), underlies the improvement seen in their subjects.

Two other intervention studies have examined the effects of exercise training on fasting glucose and insulin when exercise was performed intermittently or continuously. In one study, 22 overweight females were allocated into either an intermittent group (two, 15 minute sessions of brisk walking at 60-75% of maximum oxygen uptake, 5 days a week), or a continuous group (one continuous bout of brisk walking at 60-75% of maximum oxygen uptake, 3 days a week) (Donnelly et al., 2000). This study found a similar reduction in insulin area under the curve values following an 18-month training programme. Fasting glucose concentration was unaffected by either group. However, as with many other studies of accumulated exercise to date, the study did not employ a control group. Another study (which did include a control group) investigated the effect of two daily walking sessions versus one continuous session (of equal intensity and energy expenditure) on glucose and insulin concentrations (Asikainen et al., 2003). The subjects were postmenopausal women and the programme lasted 15 weeks. Each training day involved an energy expenditure of 1.26 MJ (300 kcal) at 65% of maximum oxygen uptake. An equal number of women with and without hormone replacement therapy were allocated into each of three groups (one or two daily walking groups and a control group). Fasting glucose concentrations and glucose concentrations during a 2-hour glucose tolerance test were reduced in both exercise groups in comparison to the control group. Insulin concentrations were unaffected by the exercise programmes.
One previous study has compared the effects of short and long bouts of exercise on body composition in overweight women (Schmidt et al., 2001). In a controlled trial, participants were allocated into four groups; a) 30 minutes of continuous exercise; b) two 15 minute bouts of exercise; c) three 10 minute bouts of exercise; d) a nonexercise control group. Exercise intervention groups performed aerobic exercise using the Schwinn AirDyne (combined arms and legs exercise machine) at 75% of their heart rate reserve, 3 to 5 days a week for 12 weeks. This well-controlled study found a reduction in body weight, body mass index (BMI), sum of skinfolds, and sum of circumferences in all exercise intervention groups in comparison to the control group. However, it is difficult to conclude that training per se contributed to enhanced body composition since subjects were asked to adhere to a self-monitored weight loss diet (energy intake = 80% of resting energy expenditure) and this may have been partly responsible for the weight loss.

In summary, the majority of intervention studies have reported that regular accumulated exercise confers a positive benefit on fitness, blood lipids, blood pressure, blood glucose, insulin and body composition. However, existing studies need to be interpreted with caution. Most of the intervention studies on accumulated exercise have used self-reported exercise and several studies have not included a control group. Hence, randomised, controlled laboratory-based intervention studies are needed to support the recommendations of recent physical activity guidelines (Pate et al., 1995; U.K. Department of Health, 2004; U.S. Department of Health and Human Services, 1996) with regard to the benefit of accumulating exercise for health.
2.4 The influence of accumulated physical activity on postprandial triacylglycerol concentrations

Since Zilversmit (1979) proposed that atherosclerosis was a postprandial phenomenon, growing evidence has supported the notion that the impaired clearance of triacylglycerol-rich lipoproteins, namely chylomicrons and very low density lipoproteins (VLDL), is a strong potential risk factor for coronary heart disease (Karpe and Hamsten, 1995; Simpson et al., 1990). Measurement of fasting triacylglycerol concentration does not necessarily provide the best index of triacylglycerol metabolic capacity, as the major catabolic pathway of triacylglycerol-rich lipoproteins occurs during the postprandial state (Weintraub et al., 1996). Moreover, people spend a majority of their lifetime, up to three-quarters of each day, in the postprandial state. Thus, repeated daily episodes of exaggerated postprandial lipaemia, and prolonged residence in the circulation of triacylglycerol-rich lipoproteins is now considered an emerging risk factor for cardiovascular disease (Cohn, 1998; Stanner, 2005). This notion is supported by the findings of case-control studies, which reveal that postprandial plasma triacylglycerol concentrations are higher in coronary heart disease patients than in healthy control subjects (Patsch et al., 1992; Groot et al., 1991). It is therefore important to study any therapy which may be effective in reducing postprandial triacylglycerol concentrations.

Recent reviews have indicated that aerobic exercise has favourable effects on the attenuation of elevated postprandial triacylglycerol concentrations (Gill and Hardman, 2003; Petitt and Cureton, 2003). The key determinant of this exercise-induced triacylglycerol lowering effect may be the total energy expenditure during exercise (Petitt and Cureton, 2003). The mechanisms underlying the reduction of triacylglycerol
in response to exercise may possibly be mediated through an increase in lipoprotein lipase activity, the rate-limiting enzyme for triacylglycerol-rich lipoprotein catabolism (Seip and Semenkovich, 1998) and/or a reduction in secretion of hepatic VLDL-triacylglycerol (Gill et al., 2001).

The partitioning of exercise sessions throughout the day may be applicable for sedentary populations who might find it easier to perform short bouts of exercise rather than one long bout due to their low physical fitness levels. Similar reductions in postprandial triacylglycerol concentrations were found from walking on a treadmill for one long session (180 minutes) of low intensity exercise (32% of maximum oxygen uptake) and one short session (90 minutes) of moderate intensity exercise (63% of maximum oxygen uptake) with equivalent energy expenditure (Tsetsonis and Hardman, 1996b). This suggests that total energy expenditure may be the key to lowering postprandial triacylglycerol concentration and supports the hypothesis that accumulating activity in very short bouts may be effective in this regard provided that sufficient energy is expended.

To the author's knowledge, three studies have compared the effect of exercise performed in short bouts and in one continuous bout on postprandial triacylglycerol concentrations. Gill et al (1998) have shown that in healthy men there was a comparable reduction in postprandial plasma triacylglycerol concentrations after three 30 minute bouts of running during one day versus and one continuous 90 minute run of equal intensity (60% of maximum oxygen uptake). In percentage terms, the total postprandial plasma triacylglycerol response to a standardised fat meal measured over a 6-hour observation period was reduced by 18.1% and 17.7% compared with the
control trial in the continuous exercise trial and intermittent exercise trial respectively. This study concluded that exercise-induced reductions in postprandial plasma triacylglycerol concentrations were due to enhanced triacylglycerol removal rather than lowered hepatic VLDL-triacylglycerol secretion. This conclusion was based on the fact that postprandial total non-esterified fatty acid (NEFA) concentrations were similar between trials in this study and the production rate of hepatic VLDL-triacylglycerol is largely dependent on the rate of NEFA supply (Frayn, 1998).

Murphy et al (2000) extended research on accumulated activity and postprandial lipaemia. In this study subjects performed 30 minutes of walking either in one continuous bout before breakfast or in three short (10 minute) bouts before breakfast, lunch and an early evening meal. The exercise intensity was the same on both trials (60% of maximum oxygen uptake). Both patterns of exercise reduced postprandial plasma triacylglycerol concentrations to a similar extent. Moreover, the greatest reductions in triacylglycerol concentrations were observed in the late afternoon/early evening. Since plasma NEFA concentrations did not differ significantly between control and exercise trials, the authors concluded that increased lipoprotein lipase activity was the most likely explanation for the reductions in postprandial triacylglycerol concentrations during the exercise trials. This is supported by the finding of a delayed increase in lipoprotein lipase activity after exercise (Gre.iwe et al., 2000; Kiens and Richter, 1998) which coincides with the finding that the greatest reductions in triacylglycerol occurred in the late afternoon/early evening in the study of Murphy et al (2000).

A recent study has shown that intermittent short bouts of exercise (10 minutes × 3, 20
minutes recovery between bouts) reduces incremental plasma triacylglycerol concentrations for 8 hours postprandially in healthy young males and females (Altena et al., 2004). However, this study did not observe the reduction in incremental plasma triacylglycerol concentrations in continuous exercise (30 minutes) despite similar energy expenditure (1 MJ = 245 kcal) between the intermittent and continuous exercise trials. This study concluded that energy expenditure may not be the only determinant of postprandial triacylglycerol responses, and the authors speculated that excess post-exercise oxygen consumption may be one of factors responsible for reduction in postprandial triacylglycerol concentrations. Further investigation into this mechanism is required. Moreover, there was no control of menstrual cycle phase in this study and this is a confounding factor since one study has shown that postprandial plasma triacylglycerol responses are lower in the luteal phase than the follicular phase of the menstrual cycle (Gill et al., 2005).

The reports from these three studies show that accumulated (or intermittent) exercise is as effective as continuous exercise session in reducing postprandial triacylglycerol concentrations. Two studies have employed a total exercise duration of 30 minutes (Altena et al., 2004; Murphy et al., 2000). However, none of these studies employed exercise bouts lasting less than 10 minutes. It is not clear that whether accumulating 30 minutes of activity in bouts lasting less than 10 minutes will lower postprandial triacylglycerol concentrations.

To date, only one study has examined postprandial lipaemia after the accumulation of exercise in short bouts on multiple days (Altena et al., 2006). In the study, eighteen untrained (7 males and 11 females) subjects were randomly assigned to either
intermittent or continuous aerobic exercise training for 4 weeks. The intermittent exercise training programme involved three, 10-minute bouts of jogging (60% of maximum oxygen uptake, 75% of heart rate max) separated by 20 minutes of rest on 5 days a week. The continuous exercise training involved one, 30 minute jog at 60% of maximum oxygen uptake (75% of heart rate max) 5 times a week. There was no training-induced lowering of postprandial plasma triacylglycerol concentrations when measurements were made at least 48 hours after the last training session. This finding is consistent with previous studies involving brisk walking (Aldred et al., 1995) and running (Herd et al., 1998) when measurements were made 48 hours and 60 hours after the last training session, respectively. This may explain why exercise-induced lowering of postprandial triacylglycerol concentration is short-lived as has been reported previously (Zhang et al., 2004), illustrating the importance of frequent physical activity.

2.5 The influence of accumulated physical activity on resting blood pressure

Epidemiological data shows that high blood pressure is associated with an increased risk of coronary heart disease (Andersson et al., 1998). Indeed in the United Kingdom, 25% of hypertensive patients have a history of angina pectoris, myocardial infarction, or both in the United Kingdom (U.K. Department of Health, 1999). Therefore, hypertension (defined as a systolic blood pressure of 140 mm Hg or over, and/or a diastolic blood pressure of 90 mm Hg or over) is also a major coronary heart disease risk factor along with hyperlipidaemia. This indicates a need for multi-factorial approaches to reduce the risk of cardiovascular disease.

Exercise has been shown to reduce blood pressure and is recommended as an
alternative for pharmacological intervention (Pescatello et al., 2004). In a meta-analysis of 54 randomised controlled studies which examined the effect of chronic aerobic training on resting blood pressure, average reductions in resting systolic and diastolic blood pressure were 3.8 mm Hg and 2.8 mm Hg respectively (Fagard, 2001). Similarly, acute exercise was effective in reducing both resting systolic and diastolic blood pressure on average by 15 mm Hg and 4 mm Hg respectively for several hours in hypertensive individuals (Pescatello and Kulikowich, 2001). With reference to the blood pressure lowering effect of aerobic training, the reduction was more pronounced in hypertensive than in normotensive individuals (Pescatello et al., 2004). It has been suggested that moderate intensity exercise (<70% of maximum oxygen uptake) is more effective in reducing blood pressure than high intensity exercise (>70% of maximum oxygen uptake). However, opinion is divided as to the optimum ingredients of a training programme for lowering blood pressure (Pescatello et al., 2004).

Acute exercise lowers resting blood pressure, this effect may last for several hours and is known as postexercise hypotension (Thompson et al., 2001). Therefore, accumulating short bouts of exercise throughout the day may be an ideal antihypertensive therapy.

2.5.1 Accumulated physical activity and blood pressure: studies of acute exercise
To date, only three studies have examined the acute effect of accumulating exercise on blood pressure (Hagberg et al., 1987; Kaufman et al., 1987; Padilla et al., 2005). The study by Kaufman et al (1987) demonstrated that five, 10-minute bouts of treadmill walking (3 minutes interval between bouts) reduces systolic and diastolic blood
pressure 15 minutes post-exercise. Moreover, systolic blood pressure remained lower 60 minutes post-exercise in normotensive and hypertensive individuals. The authors concluded that the lowering of blood pressure after exercise was possibly due to a reduction in stroke volume subsequent to an increase in peripheral vasodilation. However, they had limited evidence to support this and the study did not include a control group.

The study by Hagberg et al (1987) investigated the mechanism underlying postexercise hypotension. Hypertensive individuals performed three, 15-minute bouts of treadmill exercise at 50% of maximal oxygen uptake with 3 minute intervals between bouts. A significant reduction in systolic blood pressure was observed 20 minutes into the post-exercise recovery period and this reduction remained for up to 60 minutes post-exercise. This study also demonstrated a reduction of cardiac output (estimated by using a computer version of the carbon dioxide rebreathing technique) and stroke volume (calculated as the quotient of cardiac output and heart rate) during a 60 minute recovery period. In a separate age-matched hypertensive control group, no changes in systolic blood pressure, cardiac output and stroke volume were observed during an equivalent period of rest.

More recently, Padilla et al (2005) investigated the effects of accumulated lifestyle physical activity on ambulatory blood pressure. Each group of 9 normotensive, 10 prehypertensive and 10 hypertensive individuals performed various lifestyle activities (i.e. splitting logs, mowing the lawn, digging/spading, tilling, raking, laying sod, and brisk walking) throughout the day. In the prehypertensive and hypertensive groups, a reduction of ambulatory systolic blood pressure was observed following accumulated
activity and this remained for up to 6 hours in the prehypertensive group and up to 8 hours in the hypertensive group compared with the non-exercising control group.

Although the studies mentioned here were designed to simulate intermittent activity and to investigate the mechanism underlying postexercise hypotension, none of these studies investigated the influence of very short bouts of exercise with longer interval periods. Therefore, a worthwhile area for future study is the influence of the accumulation of short bouts of exercise interspersed with longer recovery periods on blood pressure. This may have practical implications for anti-hypertensive therapy. Nonetheless, the findings from the study by Padilla et al (2005) are particularly useful since ambulatory blood pressure was assessed and the majority of studies have measured blood pressure at rest which may not truly reflect a real life setting.

2.5.2 Accumulated physical activity and blood pressure: studies of exercise training

Several studies have investigated the effects of accumulated exercise training on resting blood pressure. One example is the study by Staffileno et al (2001) which examined the effect of accumulating short bouts of exercise on resting blood pressure in 18 postmenopausal, sedentary, untreated hypertensive women. Subjects were randomly assigned to an 8-week intermittent moderate-intensity activity programme (performing selective activities, such as walking for 10 minutes, 3 times a day, 5 days a week at 50-60% of heart rate reserve) or to a control programme. In comparison with the control group, the intermittent exercise group reduced their resting systolic and diastolic blood pressures by 8 mm Hg and 5 mm Hg respectively at the end of training programme. In this independent group design, the differences remained after adjusting
Another study investigated whether lifestyle physical activity decreases blood pressure to a similar extent as structured exercise (Dunn et al., 1997). In the lifestyle physical activity group, participants were asked to accumulate at least 30 minutes of moderate-intensity physical activity (i.e. walking, playing sports with children, shopping) on most days of the week for 6 months. The structured exercise group received a ‘traditional’ exercise prescription (American College of Sports Medicine Position Stand, 1990), performing exercise 20-60 minutes at a time at an intensity of 50-85% of maximum oxygen uptake for 3 to 5 days a week. After 6 months, both groups showed significant reductions in resting systolic and diastolic blood pressure. This was the first randomised, clinical trial, to demonstrate that lifestyle activity is equally effective in reducing blood pressure as structured exercise.

A study by Coleman et al (1999) investigated the effects of accumulating exercise bouts shorter than 10 minutes per session on blood pressure. Participants were randomly assigned to three different patterns of brisk walking: a) 30 minutes of continuous walking, b) three, 10-minute bouts of walking, c) 30 minutes of accumulated walking with each bout at least 5 minutes in duration. Walking was performed on six days per week. Self-report data revealed that resting systolic blood pressure was reduced in all groups by 5, 2, and 7 mm Hg for groups a, b, and c respectively at the end of programme (16 weeks) and this reduction was maintained at follow-up (32 weeks). This finding is important since accumulating exercise bouts less than 10 minutes in duration may be an attractive/manageable exercise strategy for previously sedentary people. However, this study did not employ a control group.
which limits the validity the findings. Moreover, the timing of blood pressure measurements was not mentioned in this study, thus it is unclear whether the reductions in blood pressure were due to the acute or chronic effects of exercise.

The mechanism responsible for the lowering of blood pressure after a single bout of exercise may be a reduction in total peripheral resistance through the reduction in sympathetic activity and enhanced peripheral vasodilation (Kenney and Seals, 1993). The chronic, training-induced lowering of blood pressure may be due to changes in the vascular structure through increased lumen diameter and improved total systemic arterial compliance (Cameron and Dart, 1994). For specific details regarding the mechanisms by which blood pressure may be reduced after acute exercise and chronic training, readers are referred to relevant review articles (Hamer, 2006; Kenney and Seals, 1993).

2.6 Exercise and C-reactive protein

C-reactive protein is a marker for inflammation. It is synthesised primarily in the liver and its activity is stimulated by interleukin-6, a proinflammatory cytokine (Castell et al., 1990). C-reactive protein exerts a protective role in response to tissue injury, but it also initiates inflammatory lesions (Pepys, 1981). C-reactive protein has received substantial attention in recent prospective studies (Ridker et al., 1997; Ridker et al., 2000), which demonstrate that elevated C-reactive protein is associated with increased risk of future cardiovascular disease. The predominant view now is that atherosclerosis develops not only due to the accumulation of lipids in arteries but also through inflammation which damages the vascular endothelium (Libby, 2002).
Cross-sectional studies demonstrate that there is an inverse relationship between the volume of regular physical activity performed and resting C-reactive protein concentrations (for a review of these see, Kasapis and Thompson, 2005). However, most cross-sectional studies have not controlled for confounding factors including smoking status, medical history, and medication/supplement use, which may affect C-reactive protein concentrations. In addition, physical activity is self-reported in many, if not all, studies and many studies have failed to adjust for body weight. Nonetheless, one cross-sectional study which did control for potential confounding factors including body weight has shown that individuals with high cardiorespiratory fitness have lower C-reactive protein concentrations compared with individuals classified as having low level of cardiorespiratory fitness (Church et al., 2002). On the basis of these findings physical activity intervention may reduce C-reactive protein concentrations and therefore help to prevent cardiovascular disease.

Kasapis and Thompson (2005) reviewed the acute effect of strenuous exercise on C-reactive protein concentrations. The findings of all previous studies are consistent in showing that there is a significant increase in C-reactive protein concentrations measured 24-48 hours following a triathlon (Fallon, 2001; Taylor et al., 1987) or a marathon race (Siegel et al., 2001; Weight et al., 1991). This short-term and transient increase in C-reactive protein may be related to the duration of exercise. It has been shown that an increase in C-reactive protein concentration was related to an increase in marathon duration (Strachan et al., 1984). Moreover, performing 45 minutes of treadmill walking did not increase C-reactive protein concentrations measured 24 hours post-exercise in sedentary, overweight men (Murtagh et al., 2005). From a public health perspective, the finding of no alteration in C-reactive protein concentration after
moderate-intensity exercise may have clinical implications, suggesting that moderate-intensity exercise may be preferential to high-intensity exercise from the point of view of reducing cardiovascular disease risk.

Conversely, regular physical activity has been shown to enhance anti-inflammatory effect (Petersen and Pedersen, 2005). Recent studies have reported that chronic exercise training lowers C-reactive protein concentrations (Kondo et al., 2006; Lakka et al., 2005; Mattusch et al., 2000; Smith et al., 1999; Tisi et al., 1997). In a large, well-controlled study, 20 weeks of standardised exercise training reduced C-reactive protein concentrations in sedentary, healthy white and black men and women with high baseline C-reactive protein profiles (Lakka et al., 2005). The exact mechanism underlying the reduction in C-reactive protein through regular physical activity is not known, but may involve in several factors, including a decrease in inflammatory cytokine production, improved endothelial function and an increase in antioxidant defenses (Kasapis and Thompson, 2005). However, other studies have produced conflicting results by suggesting that C-reactive protein concentrations are not reduced after exercise training (Duncan et al., 2004; Marcell et al., 2005). These discrepancies may be due to a variety of factors including differences in: a) training volume, b) baseline C-reactive protein concentrations, c) the health status of the subjects, d) weight loss or lack of it. Further studies are required to clarify the influence of exercise training on C-reactive protein concentration.

The standardisation of preanalytical and analytical assay procedures also needs to be established in the future. Different studies employ different assays which may have contributed to the discrepant results (Ledue and Rifai, 2003). In addition, the
measurement of high-sensitivity C-reactive protein to obtain a sufficient detection range is important not only for diagnostic purposes, but also for research purposes in order to quantify changes in C-reactive protein concentrations in response to exercise.

2.7 Exercise and ghrelin

Ghrelin, a novel gastrointestinal hormone, was identified by Kojima et al and functions as a growth hormone releasing peptide (Kojima et al., 1999). Ghrelin is composed of 28 amino acids with a unique acylated structure, in which the serine 3 residue is modified by n-octanoic acid (Hosoda et al., 2003; Kojima et al., 1999). This n-octanoyl modification is essential for the biological regulation of the activity of ghrelin (Kojima et al., 1999; Matsumoto et al., 2001). Ghrelin is predominately secreted from the stomach (Ariyasu et al., 2001), and to a lesser extent the pituitary and hypothalamus (Korbonits et al., 2001).

Plasma ghrelin concentrations rise prior to meals and decrease after meals suggesting that ghrelin is involved in the acute regulation of appetite (Shiiya et al., 2002; Cummings et al., 2001). Ghrelin has other physiological roles but since these are not directly related to this thesis the interested reader is directed to relevant review papers (Korbonits et al., 2004; Muccioli et al., 2002). Since ghrelin is involved in appetite regulation and hence energy balance it is of interest to assess any effects exercise might have on plasma ghrelin concentrations.

To the author’s knowledge, six studies have examined the influence of an acute bout of aerobic exercise on total plasma ghrelin concentration (Burns et al., in press; Dall et al., 2002; Kallio et al., 2001; Kraemer et al., 2004a; Schmidt et al., 2004; Zoladz et al., 2004; Zoladz et al., 2005).
The findings of these studies are consistent and indicate that a single bout of cycling (Dall et al., 2002; Kallio et al., 2001; Zoladz et al., 2005) or running (Burns et al., in press; Kraemer et al., 2004a; Schmidt et al., 2004) has no influence on total plasma ghrelin concentration. If ghrelin is regulated by short-term energy balance, its concentration would be expected to increase following an exercise induced energy deficit. It can be speculated that the lack of change in total plasma ghrelin concentrations may be due to an inadequate volume, intensity or duration of exercise employed in previous studies. However, Burns et al (in press) employed a quite strenuous exercise protocol (74% of maximum oxygen uptake for 1 hour), therefore such speculation may be unjustified.

Conversely, in a study involving resistance exercise, total plasma ghrelin concentrations were significantly decreased immediately post-exercise and during the first hour of post-exercise recovery in young healthy males after intense concentric resistance exercise (Kraemer et al., 2004b). It is not clear why such discrepant results are observed between aerobic and resistance exercise since the precise mechanism involved in circulating ghrelin in response to exercise has not been elucidated. However, many of the studies cited here lacked a control group. Total ghrelin concentrations fluctuate throughout the day (Cummings et al., 2001; Pumell et al., 2003) even in the absence of food intake (Natalucci et al., 2005). Therefore, the lack of a control group in the previous studies (Dall et al., 2002; Kallio et al., 2001; Kraemer et al., 2004b; Schmidt et al., 2004; Zoladz et al., 2005) makes it difficult to interpret the results. More randomised controlled studies are required to gain a better understanding of the interactions between exercise and plasma ghrelin.
Taken together the results from studies on exercise and plasma ghrelin suggest exercise has little, if any, influence on ghrelin. However, all of the available studies are limited by the fact that they have measured total ghrelin. Ghrelin exists in two forms: acylated (active) and des-acylated (inactive). Des-acylated ghrelin has limited, if any, biological action despite comprising 94% of total ghrelin (Yoshimoto et al., 2002). There is therefore a need to evaluate the influence of exercise on active ghrelin since this is the form of ghrelin which is thought to be largely responsible for stimulating appetite (Kojima et al., 1999; Hosoda et al., 2000; Matsumoto et al., 2001).

2.8 Summary

The purpose of this chapter was to present an overview of the current knowledge relating to accumulated physical activity and cardiovascular disease risk. In particular, the effects of accumulated exercise on postprandial triacylglycerol metabolism and resting blood pressure were addressed. With respect to the issue of accumulation, several recent studies support the concept that accumulated physical activity is beneficial to health. However, limited evidence is available regarding the effects on health of accumulating very short bouts of exercise i.e. bouts lasting less than 10 minutes in duration. Moreover, no studies have investigated the effects of accumulating short (< 10 minute) bouts of exercise on postprandial triacylglycerol concentrations, resting blood pressure, high-sensitivity C-reactive protein concentrations and acylated ghrelin concentrations. Intervention studies are required to address these issues. The studies reported in this thesis involve acute exercise interventions examining the effects of accumulating short bouts of exercise throughout a single day on various markers for health/disease risk. It is hoped that such information will help to shape future guidelines related to exercise and health.
CHAPTER 3
GENERAL METHODS

This chapter describes the experimental procedures employed during the studies described in this thesis. The majority of experimental procedures are common to each experiment. All studies were conducted under the approval of Loughborough University's Ethical Advisory Committee.

3.1 Subject recruitment

Male subjects were recruited from within Loughborough University and the local area by word-of-mouth and/or poster advertising. Volunteers received written information (for an example see, Appendix A) regarding the study including potential risks and discomforts and were given the opportunity to ask questions regarding the nature of the study before signing a statement of informed consent (for an example see, Appendix B). Following informed consent, each subject underwent a confidential health screening process using the University's health questionnaire (Appendix C). Subjects also completed a physical activity questionnaire (Appendix D) which examined their activity levels, and their arterial blood pressure was measured (section 3.5). To minimise risks, subjects were only recruited if they met the following criteria:

a) were non-smoking
b) were normally active
c) were free of known cardiovascular disease or abnormalities, acute illness or active, chronic systemic disease
d) were not taking any medication known to influence lipid or carbohydrate metabolism
e) had no orthopaedic or muscular contraindications to treadmill walking or running
f) had no known dyslipidaemia
g) had non-extreme dietary habits
h) had resting arterial blood pressure <140/90 mmHg
i) had a BMI <35 kg/m².

3.2 Anthropometry

Anthropometric measurements were made in all studies described in this thesis. Skinfold thickness was measured in each study except that reported in Chapter 4.

3.2.1 Height

Height was measured using a fixed-wall stadiometer (Seca, Hamburg, Germany). Subjects stood barefoot with their heels together against a wooden back plate. Arms were relaxed loosely by their sides. The head was placed in the Frankfort Plane i.e. a horizontal line between the lower orbits of the eyes and the external auditory meatus. The stadiometer head plate was lowered onto the top of the head and then height was recorded to the nearest 0.1 cm.

3.2.2 Weight

Weight was measured, to the nearest 0.01 kg, using a balance beam scale (Avery, Birmingham, United Kingdom). Subjects wore light clothing and removed their shoes while being weighed.

3.2.3 Body mass index

Body mass index was calculated as weight in kilograms divided by the square of height
3.2.4 Skinfold measurements

Skinfold thickness was measured using callipers (John Bull, British Indicators, West Sussex, United Kingdom). The measurements were taken at the following sites on the right hand side of the body with the subject standing:

a) Chest – this measurement was made one-half of the distance between the anterior axillary line and the nipple.

b) Abdomen – this measurement was made 2 cm to the right of the umbilicus.

c) Thigh – this measurement was made on the anterior midline of the thigh, midway between the proximal border of the patella and the inguinal crease.

Each skinfold was lifted by the experimenter’s left hand thumb and index finger. The skinfold callipers were placed 1 cm above the site of measurement. The measurement was taken after 1-2 seconds of calliper pressure while still pinching the skinfold. Each site was measured in triplicate. The skin was allowed to regain its normal texture and thickness between measurements. The average of the three measurements for each site was calculated and used to represent the skinfold thickness for that site. The sum of skinfold measurements was used to estimate body density using a three sites formula (Jackson and Pollock, 1978) and body fat percentage then estimated using the Siri equation (Siri, 1956).

3.2.5 Waist circumference

Waist circumference was measured with an inelastic polyfibre tape measure (Hoechstmass Balzer GmbH, Sulzbach, Germany) placed directly on the skin while the
subject stood balanced on both feet, with the feet touching each other and both arms hanging freely. The measurement was taken at the end of expiration. Waist circumference was determined as the widest part of the torso between the xiphoid process of the sternum and the iliac crest.

3.3 Heart rate measurement
Heart rate was measured during the preliminary exercise tests and main trials (Chapters 4, 5, 6, 7, and 8 Parts II and III) using short range telemetry (Polar A3, Polar Electro, Kempele, Finland).

3.4 Ratings of perceived exertion
Ratings of perceived exertion (RPE) were used to obtain each individual’s perception of exercise intensity during the preliminary exercise tests and main exercise trials (Chapters 4, 5, 6, 7, and 8) using the Borg Scale (Borg, 1973), with numbers rating from 6 (no exertion at all) to 20 (maximal exertion).

3.5 Arterial blood pressure measurement
Arterial blood pressure was measured by auscultation using a mercury sphygmomanometer (Accoson freestyle stand 0042, CardioHinetics, Salford, United Kingdom) (Chapter 8 Part I) or a random-zero sphygmomanometer (Hawksley Mk. II, Hawksley and Sons Ltd., Sussex, United Kingdom) (Chapter 8 Parts II and III), according to the guidelines described by the British Hypertension Society (Ramsay et al., 1999; Williams et al., 2004). Arterial blood pressure was measured at the health screening stage and during each main study. Subjects were seated on a chair for 5 minutes before measurements (except for measurements immediately after exercise.
during the session). Two measurements were taken at each time point and the mean of these values recorded.

3.6 Exercise tests

Exercise tests were performed on a motorised treadmill (RUNRACE, Technogym, Gambettola, Italy). All subjects were familiarised with treadmill walking/running prior to testing.

3.6.1 Submaximal exercise testing

A 16-minute, four-stage submaximal, treadmill test was conducted to determine the relationship between walking/running speed and oxygen uptake. Initial treadmill speed was set between 6 and 8 km/h depending upon each subject's fitness level. The treadmill was level throughout the test. Speed was increased by between 1 and 2 km/h every 4 minutes—depending upon each subject's fitness level. Expired air samples were collected during the last minute of each test stage. Heart rate was recorded during the expired air collections using short-range telemetry. Ratings of perceived exertion were assessed periodically during the tests using the Borg scale (Borg, 1973). A linear regression equation was used to calculate the relationship between walking/running speed and oxygen uptake.

3.6.2 Maximal exercise testing

Maximum oxygen uptake was measured directly using an incremental uphill protocol at a constant speed until the subjects reached volitional fatigue (Taylor et al., 1955). The initial incline of the treadmill was set at 3.5% for this test. Thereafter treadmill gradient was increased by 2.5% every 3 minutes. Expired air, heart rate and RPE were
collected from 1:45 to 2:45 minutes of each 3-minute stage and throughout the final minute of the test. The final expired air collection was started when subjects felt they could run for only one more minute. The highest oxygen uptake achieved during the test (usually the final collection) was accepted to be the subject’s maximum oxygen uptake. Strong verbal encouragement was given to subjects throughout the test.

3.6.3 Determination of self-paced brisk walking

In the study reported in Chapter 6, walking speed was self-selected during several minutes of walking on the treadmill. ‘Brisk’ walking was defined as feeling slightly out of breath while walking but still able to hold a conversation.

3.7 Oxygen uptake and carbon dioxide production measurement

Expired air samples were collected into Douglas bags (Plysu Protection Systems, Milton Keynes, United Kingdom). Oxygen uptake and carbon dioxide production were determined from these expired air samples using a paramagnetic oxygen analyser and an infra-red carbon dioxide analyser respectively (Series 1400; Servomex, Crowborough, East Sussex, United Kingdom). These analysers were calibrated prior to analysis using gases of known concentration. Expired air volumes were measured using a dry gas metre (Harvard Apparatus, Edenbridge, Kent, United Kingdom) and corrected to standard temperature and pressure (dry). The dry gas metre was calibrated using a 3 litre calibration syringe (Series 5530; Hans Rudolph Inc., Kansas City, Missouri, U.S.A.). Oxygen consumption and carbon dioxide production values were used to calculate energy expenditure (Frayn, 1983).
3.8 Experimental protocol – main trials

Subjects underwent two, 2-day trials (Chapters 4 and 8 Part I), three, 2-day trials (Chapters 5, 6, and 8 Parts II and III), and two, 1-day trials (Chapter 7) separated by at least an interval of 7-day in a randomised repeated-measures design. Although full details of the experimental protocol are presented in the methods section of each experimental chapter, brief protocol details of each experimental study are described here.

In Chapters 4 and 8 Part I, subjects rested (no exercise) or performed multiple 6 minute running bouts (30 minutes rest between each) until they had accumulated an energy expenditure of 4.2 MJ (1000 kcal) on day 1. Subjects rested and consumed test meals (section 3.9) for breakfast and lunch on day 2. Blood pressure was measured throughout days 1 and 2 (Chapter 8 Part I). Venous blood samples were obtained throughout day 2 (Chapter 4).

In Chapters 5 and 8 Part II, subjects rested (no exercise) or ran at 70% of maximum oxygen uptake in either ten, three-minute bouts (30 minutes rest between each), or one continuous 30-minute bout on day 1. Subjects rested and consumed test meals for breakfast and lunch on day 2. Blood pressure was measured throughout days 1 and 2 (Chapter 8 Part II). Venous blood samples were obtained throughout day 2 (Chapter 5).

In Chapters 6 and 8 Part III, subjects rested (no exercise) or walked at self-selected brisk pace in either ten, three-minute bouts (30 minutes rest between each), or one continuous 30-minute bout on day 1. Subjects rested and consumed test meals for breakfast and lunch on day 2. Blood pressure was measured throughout days 1 and 2.
(Chapter 8 Part III). Venous blood samples were obtained throughout day 2 (Chapter 6).

In Chapter 7, subjects rested (no exercise) or ran at 70% of maximum oxygen uptake in six, 5-minute bouts (85 minutes rest between each) whilst venous blood samples were obtained throughout the day.

3.9 The test meals

In all experimental studies described in this thesis, subjects consumed test meals for breakfast and lunch. Each test meal was identical. The test meal consisted of white bread, Cheddar cheese, butter, mayonnaise, potato crisps, whole milk and milkshake powder. The meal was prescribed according to body mass and provided 0.69 g fat, 0.95 g carbohydrate, 0.31 g protein, and 46 kJ (11 kcal) energy per kilogram body mass. The test meal quantity and its macronutrient composition for a subject of 70 kg are shown in Table 3.1 and Table 3.2. Subjects were asked to consume each meal within 20 minutes. Subjects consumed water *ad libitum* during the first trial and the volume ingested was replicated in subsequent trials (Chapters 4, 5, 6 and 8). In Chapter 7, subjects consumed water *ad libitum* during each main trial since the trials were randomised and each trial was conducted over 1-day (exercise/control and postprandial testing was conducted on the same day), but the volume ingested was recorded.
Table 3.1 Quantities of each ingredient of the test meal, provided per kg body mass

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity (per kg body mass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White bread (g)</td>
<td>1.10</td>
</tr>
<tr>
<td>Cheddar Cheese (g)</td>
<td>0.47</td>
</tr>
<tr>
<td>Butter (g)</td>
<td>0.17</td>
</tr>
<tr>
<td>Potato Crisps (g)</td>
<td>0.43</td>
</tr>
<tr>
<td>Whole Milk (g)</td>
<td>2.14</td>
</tr>
<tr>
<td>Mayonnaise (g)</td>
<td>0.17</td>
</tr>
<tr>
<td>Milkshake Powder (g)</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Table 3.2 Macronutrient composition of the test meal

<table>
<thead>
<tr>
<th></th>
<th>Quantity (per kg body mass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ)</td>
<td>46.1</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>0.69</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>0.90</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>0.31</td>
</tr>
<tr>
<td>Fat (% energy)</td>
<td>56.0</td>
</tr>
<tr>
<td>Carbohydrate (% energy)</td>
<td>33.0</td>
</tr>
<tr>
<td>Protein (% energy)</td>
<td>11.0</td>
</tr>
</tbody>
</table>

3.9.1 Standardisation prior to the test meals

In Chapters 4 and 8 Part I, subjects weighed and recorded all food and drink consumed during the day before their first trial and on day 1 of their first trial. Subjects were asked to replicate their intake from their first trial on their second trial. In Chapters 5, 6 and 8 Parts II and III subjects weighed and recorded all food and drink consumed for two days prior to any of the main trials. Subjects then consumed identical amounts of
the same food and drink prior to each of the main trials. In Chapter 7, subjects weighed and recorded all food and drink consumed 2 days prior to their first trial. Subjects were asked to consume identical amounts of the same food and drink prior to their second trial. The reason for the difference in dietary preparation between Chapters 4 and 8 (Part I) and Chapters 5, 6 and 8 (Parts II, III) was that the author wanted to standardise diet carefully. The author did not consider carefully that the energy intake of the evening meal (day 1) prior to the postprandial testing (day 2) might be affected by the trial order when conducting the first study (Chapters 4 and 8 Part I). Therefore, in subsequent studies (Chapters 5, 6 and 8 – Parts II, III), subjects were asked to record all food and drink consumed for two days prior to any of the main trials. In Chapter 7, since the trials were conducted over 1-day the order of the trials would not affect energy intake during the 2-day recording period prior to each main trial.

Subjects refrained from drinking alcohol whilst they were recording their diet. In addition, subjects were asked to remain inactive on the day before day 1 of each trial and throughout the main trials (other than the exercise performed as part of the experiment). In Chapter 7, subjects were asked to remain inactive on two days before the start of each main trial and throughout the main trials (other than the exercise performed as part of the experiment). Subjects were instructed to come to the laboratory after a 10-hour overnight fast (no food or drink except water).

3.10 Blood sample collection
Venous blood samples were obtained via an indwelling cannula (Venflon, Becton Dickinson, Helsingborg, Sweden) placed in an antecubital vein. The first 3 mL of blood withdrawn was always discarded and 10 mL of non-heparised saline (0.9% v/w,
B.Braun Medical, Sheffield, United Kingdom) was used to flush the cannula after each blood sample collection. All blood samples were obtained with subjects in a semi-supine position for 5 minutes prior to collection (except samples collected immediately after exercise as described in Chapter 7). For plasma triacylglycerol, glucose, insulin and NEFA measurements, venous blood samples were collected into pre-cooled potassium-ethlenediamine tetra-acetic acid (EDTA)-coated Monovette tubes (EDTA 1.6 mg/mL) (Sarstedt, Leicester, United Kingdom) via a multi-adapter (Sarstedt, Leicester, United Kingdom). For plasma acylated ghrelin measurement, venous blood samples were collected into pre-cooled EDTA-coated Monovette tubes (EDTA 1.6 mg/mL) with p-hydroxymercuribenzoic acid to prevent the degradation of acylated ghrelin by protease via a multi-adapter. For serum high-sensitive C-reactive protein measurement, venous blood samples were collected into pre-cooled Monovette with clot activator via a multi-adapter. At baseline and the end of observation period of each study small aliquots of blood were taken from a potassium-EDTA-coated Monovette tube for haemoglobin and haematocrit measurements (see section 3.11.1).

Blood collected into potassium-EDTA-coated Monovette tube was immediately centrifuged (GS-15R Centrifuge, Beckman Coulter, Fullerton, U.S.A.) at 4000 revolutions per minute (1968 x g) for 10 minutes at 4°C. After separation of venous samples, plasma (not less than 0.5 mL – to minimise any freeze drying effect) was dispensed into plain micro tubes (Sarstedt, Leicester, United Kingdom), and stored at -80°C for later analysis of plasma triacylglycerol, glucose, insulin and NEFA. For plasma acylated ghrelin measurement, samples were immediately centrifuged at 3500 revolutions per minute (1287 x g) for 10 minutes at 4°C. After separation of venous samples, 1 mL of plasma was dispensed into storage tubes and 100 µL of 1 M
hydrochloric acid was then added. Samples were then centrifuged at 3500 revolutions per minute (1287 x g) for 5 minutes at 4°C before transferred into plain micro tubes. The sample were then stored at -80°C for later analysis. For serum high-sensitivity C-reactive protein measurement, samples were allowed to clot for 45 minutes at room temperature and then centrifuged at 4000 revolutions per minute (1968 x g) for 10 minutes at 4°C. After separation of venous samples, serum (not less than 0.5 mL - to minimise any freeze drying effect) was dispensed into plain micro tubes, and stored at -80°C for later analysis.

3.11 Blood sample analysis

Blood sample analysis was conducted in the Exercise Biochemistry Laboratory in the School of Sport and Exercise Sciences unless otherwise stated.

3.11.1 Estimation of changes in plasma volume

Haemoglobin concentration and haematocrit were used to estimate changes in plasma volume (Dill and Costill, 1974). Haemoglobin concentration was assayed in duplicate by a cyanmethaemoglobin method using an ultraviolet-visible spectrophotometer (CECIL CE1011, Cecil Instruments Ltd., Cambridge, England). Haematocrit was assayed in triplicate using a microlitre-haematocrit centrifuge (MIKRO 20, Andreas Hettich GmbH and Co.KG, Tuttlingen, Germany). These assays were conducted in the Exercise and Health Research Laboratory in the School of Sport and Exercise Sciences, Loughborough University.

3.11.2 Spectorophotometric assays

The plasma concentrations of triacylglycerol, glucose, 3-hydroxybutyrate (Randox
laboratories, Crumlin, United Kingdom) and NEFA (Wako Chemicals, Neuss, Germany) were determined by enzymatic, colorimetric methods using a centrifugal analyser (Cobas Mira Plus, Roche Diagnostic Systems, Basel, Switzerland). These assays were conducted in the Exercise Biochemistry Laboratory in the School of Sport and Exercise Sciences, Loughborough University.

3.11.3 Radioimmunoassay

Plasma insulin concentrations were determined by a solid-phase $^{125}\text{I}$ (iodine 125) radioimmunoassay using a commercially available kit (MP Biomedicals, Orangeburg, U.S.A.). Radioactivity was measured by an automated gamma counting system (Cobra II, Packard Instrument Company, Downers Grove, U.S.A.). This assay was conducted in the Environmental Radiochemistry Research Laboratory in the Department of Chemistry, Loughborough University.

3.11.4 Enzyme-linked immuno sorbent assay

Plasma acylated ghrelin concentrations were determined by enzyme-linked immuno sorbent assay using a commercially available kit (SPI BIO, Montigny le Bretonneux, France). Serum high-sensitivity C-reactive protein concentrations were determined by enzyme-linked immuno sorbent assay using a commercially available kit (DRG Instruments GmbH, Marburg, Germany). For both plasma acylated ghrelin and serum high-sensitivity C-reactive protein, absorbance was measured by a plate reader (Opsys Microplate Reader, Dynex Technologies Inc., Franklin, U.S.A.). These assays were conducted in the Exercise and Immunology Laboratory in the School of Sport and Exercise Sciences, Loughborough University.
CHAPTER 4
ACCUMULATING SHORT BOUTS OF RUNNING EXERCISE
THROUGHOUT THE DAY REDUCES POSTPRANDIAL PLASMA
TRIACYLGLYCEROL CONCENTRATIONS

4.1 Introduction
In 1995 the Centers for Disease Control and Prevention and the American College of Sports Medicine introduced the unique concept of accumulation in their public health guidelines for physical activity. This was encapsulated in the statement that ‘every US adult should accumulate 30 minutes or more of moderate-intensity physical activity on most, preferably all, days of the week’ (Pate et al., 1995). This recommendation attained wide publicity the following year with the publication of the Surgeon General’s report on Physical Activity and Health (1996) and the concept of accumulation has been endorsed recently by the Department of Health in the UK (2004).

When the concept of accumulation was introduced it was recognised that the health benefits were ‘unproved’ (U.S. Department of Health and Human Services, 1996) and that ‘more research is needed’ to elucidate the health effects of accumulated activity (Pate et al., 1995). Recent studies have addressed this issue by examining a variety of health related outcomes (Table 2.1). The findings from these studies support the concept that accumulating activity is beneficial to health.

The majority of accumulation studies have employed exercise bouts with a minimum duration of 10 minutes (Table 2.1). This is not surprising since physical activity and
health guidelines recommend a minimum of 10 minutes per bout when accumulating activity (Pate et al., 1995; U.K. Department of Health, 2004; U.S. Department of Health and Human Services, 1996). However, if total energy expenditure is the most important factor for health benefits, as is suspected (Pate et al., 1995), then the duration of exercise should not matter provided that sufficient energy is expended. This issue is important not just from an academic standpoint, but also for practical reasons because research suggests that even amongst people not classed as sedentary, the majority do not exercise continuously for 10 minutes at a time (Whitt et al., 2003).

Postprandial lipaemia is considered an emerging risk factor for cardiovascular disease (Stanner, 2005) and a single bout of continuous aerobic exercise lowers postprandial lipaemia (Gill and Hardman, 2003). As mentioned in the review, three studies have examined the effect of accumulating exercise on postprandial plasma triacylglycerol concentrations (Altena et al., 2004; Gill et al., 1998; Murphy et al., 2000). Gill et al (1998) have shown that in healthy men there was a comparable (18%) reduction in postprandial triacylglycerol concentrations when performing three, 30 minute bouts of running throughout the day compared with one 90 minute bout of equal intensity (60% of maximum oxygen uptake). Murphy et al (2000) extended these findings by showing that walking at an intensity equivalent to 60% of maximum oxygen uptake either in one continuous session (30 minutes) or in three separate sessions (10 minutes x 3), reduced postprandial plasma triacylglycerol concentrations to a similar extent. Altena et al (2004) demonstrated that intermittent short bouts of running (10 minutes x 3, 20 minute interval between bouts) at an intensity equivalent to 60% of maximum oxygen uptake reduces incremental plasma triacylglycerol concentrations for 8 hours postprandially in healthy young adults. However, the minimum duration of any one
activity bout in these studies was 10 minutes. To the author's knowledge, no studies have examined the influence of accumulating very short (<10 minute) bouts of activity on plasma triacylglycerol concentrations after meals. Therefore, the purpose of the present study was to examine the effect of accumulating short (6 minute) bouts of running on postprandial triacylglycerol concentrations in young men.

4.2 Methods

4.2.1 Subjects

Nineteen healthy males (aged 19 to 26 years) volunteered to participate in this study. All subjects were recreationally active and had been weight stable (± 2.5 kg) for at least 3 months before the study. To minimise risks, subjects were only recruited if they met the following criteria: were non-smoking, were free of known cardiovascular disease or abnormalities, were not taking any medication known to influence lipid or carbohydrate metabolism, had resting arterial blood pressure <140/90 mmHg, and had a BMI <35 kg/m². Mean (± SEM) values for the subjects' age, height, weight, BMI, waist circumference, and maximum oxygen uptake were: 22.7 ± 0.5 y, 177.8 ± 1.5 cm, 75.9 ± 3.5 kg, 23.8 ± 0.8 kg/m², 83.3 ± 2.3 cm and 60.3 ± 2.0 mL/kg/min, respectively. Loughborough University's Ethical Advisory Committee approved the study and each subject gave a statement of informed consent.

4.2.2 Preliminary testing

Anthropometric measurements were made as described in section 3.2. Subjects then performed submaximal (section 3.6.1) and maximal (section 3.6.2) incremental exercise tests to establish the running speed required to elicit 70% of maximum oxygen uptake in the main trials.
4.2.3 Main trials

Each subject underwent two, 2-day trials: an exercise and a control trial. Two-day trials were used (control/exercise day 1, postprandial testing day 2) because skeletal muscle lipoprotein lipase activity is thought to peak >8 hours after exercise (Seip et al., 1997) and this enzyme facilitates the removal of triacylglycerol from the blood (Seip and Semenkovich, 1998). There was a seven-day gap between each trial, and the trials were performed in a randomised design (for the schematic representation of the study protocol, see Figure 4.1).
Exercise trial: subjects performed 6-minute runs (at 70% of maximal oxygen uptake) throughout the day with 30 minute rest intervals between bouts. This continued until subjects had accumulated a gross energy expenditure of 4.2 MJ (1000 kcal). This required between 9 and 15 bouts depending on the subject.

Control trial: subjects rested in the laboratory throughout the day.

**Figure 4.1** Schematic representation of the study protocol
Day 1. On the first day of each trial, subjects reported to the laboratory between 08:30 and 09:00 having eaten breakfast. For the exercise trial, subjects performed 6 minute bouts of treadmill running throughout the day with 30 minutes rest between each bout. The exercise intensity for the running bouts was set at 70% of maximum oxygen uptake as determined by the preliminary exercise tests. Heart rate was monitored during exercise (section 3.3). This pattern continued until subjects had accumulated a gross energy expenditure of 4.2 MJ (1000 kcal) (this expenditure was chosen because previous research - with continuous exercise - indicates that it is effective in lowering postprandial lipaemia (Petitt and Cureton, 2003)). This required between 9 and 15 bouts depending on the subject. The expired air samples - collected during the final two minutes of each bout of exercise - were used to determine the actual energy expenditure accumulated during exercise (section 3.7). Ratings of perceived exertion were assessed periodically during each bout of exercise using the Borg scale (Borg, 1973) (section 3.4). For the control trial, subjects rested throughout the day in the laboratory. During each trial, subjects consumed a packed lunch midway through the day (12:00-13:00). Subjects left the laboratory between 16:00 and 17:00 and they were instructed to consume an early evening meal and rest for the remainder of the evening.

Day 2. On the second day of each trial subjects reported to the laboratory at 08:00 after a 10 hour overnight fast (no food or drink except water). Subjects sat in a semi-supine position on a bed for 10 minutes following arrival at the laboratory. A cannula (Venflon, Becton Dickinson, Helsingborg, Sweden) was then inserted into an antecubital vein and a baseline blood sample was collected (section 3.10). Subjects then consumed a standardised test meal for breakfast (section 3.9). A clock was started when subjects began eating and they were required to rest (reading, watching television) in the
laboratory for seven hours following the initiation of breakfast. A second test meal (identical to the first) was consumed three hours after the initiation of the first meal. Venous blood samples were collected at hourly intervals throughout the day for the measurement of triacylglycerol, glucose, insulin and NEFA (section 3.11). Additional samples were collected at 0.5, 0.75, 3.5 and 3.75 hours for measurement of glucose and insulin.

4.2.4 Standardisation of diet and exercise

For purposes of standardisation, subjects weighed and recorded all food and drink consumed during the day before each trial and on day 1 of each trial. Subjects abstained from drinking alcohol during this time. Subjects were asked to replicate their intake from their first trial on their second trial. In addition, subjects were asked to remain inactive on the day before day 1 of each trial and throughout the main trials (other than the exercise performed as part of the experiment). Food diaries were analysed using computerised software (Comp-EAT Version 5.0, Nutrition Systems, London, United Kingdom) to determine energy intake and macronutrient content.

4.2.5 The test meals

Full details of the tests meal are given in section 3.9. The average macronutrient content of each test meal was $52.4 \pm 2.4 \text{ g fat, } 72.1 \pm 3.4 \text{ g carbohydrate, and } 23.5 \pm 1.1 \text{ g protein which provided } 3.49 \pm 0.2 \text{ MJ (834 \pm 39 kcal) of energy (56\% fat, 33\% carbohydrate and 11\% protein). All subjects consumed the test meals within 20 minutes and none of the subjects reported nausea or any gastrointestinal discomfort. Subjects consumed water } \textit{ad libitum} \text{ during the first trial and the volume ingested was replicated in the subsequent trial.}
4.2.6 Blood sample analysis

Blood samples were analysed for triacylglycerol, glucose, NEFA and insulin. Full details of the blood sample analysis are given in section 3.11. Intra-assay coefficients of variation were 0.5% for triacylglycerol, 1.0% for glucose, 1.4% for NEFA, and 7.4% for insulin. Haemoglobin concentration and haematocrit were measured at baseline and the end of observation period for estimating changes in plasma volume (Dill and Costill, 1974) (section 3.11.1).

4.2.7 Statistical analysis

Data were analysed using the Statistical Package for the Social Science (SPSS) software version 11.0 for Windows (SPSS Inc, Chicago, U.S.A.). The Shapiro-Wilk test was used to check for normality. Area under the curve values for plasma concentrations over time were calculated using the trapezium rule. Student’s $t$-tests for correlated data were used to assess trial differences between fasting plasma concentrations and area under the curve values. Two-way analysis of variance (ANOVA) with repeated measures was used to examine differences in plasma concentration responses between trials over time. Statistical significance was accepted at the 5% level. Results are presented as mean ± SEM.

4.3 Results

4.3.1 Responses to treadmill running

The total gross energy expenditure during accumulated exercise bouts was 4.2 ± 0.3 MJ (1000 ± 71 kcal). Average percentage maximum oxygen uptake and heart rate attained during the accumulated exercise bouts were 69.7 ± 0.6% and 161 ± 3 beats/min, respectively. The median RPE during accumulated exercise bouts was 12
(midway between ‘fairly light’ and ‘fairly hard’). The total accumulated exercise duration was $64.8 \pm 2.2$ minutes and the average number of exercise bouts performed was $11.1 \pm 0.3$.

4.3.2 Dietary data
Analysis of food diaries revealed that energy and macronutrient intake did not differ significantly in the 48 hours preceding day 2 of the control and exercise trials. Average energy intake for the day prior to the trials was $10.4 \pm 0.7$ MJ (2476 ± 167 kcal). Average energy intake for day 1 of the trials was $11.7 \pm 0.9$ MJ (2786 ± 214 kcal). Average dietary intake of fat, carbohydrate and protein was $88.2 \pm 8.9$ g, $359.4 \pm 27.9$ g and $105.3 \pm 9.2$ g respectively, for the pre-trial day, and $97.3 \pm 12.7$ g, $400.6 \pm 33.1$ g and $116.4 \pm 11.8$ g respectively, for day 1.

4.3.3 Plasma concentrations in the fasted state
Plasma concentrations in the fasted state prior to the test meals on day 2 of each trial are shown in Table 4.1. There was no difference in fasting plasma triacylglycerol, glucose and insulin between the exercise and control trials. Fasting plasma NEFA concentrations were higher on the exercise trial compared with the control trial.
Table 4.1 Fasting plasma concentrations of triacylglycerol, glucose, insulin and non-esterified fatty acids (NEFA) for day 2 of the exercise and control trials

<table>
<thead>
<tr>
<th>Trial</th>
<th>Triacylglycerol mmol/L</th>
<th>Glucose mmol/L</th>
<th>Insulin pmol/L</th>
<th>NEFA mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise</td>
<td>0.97 ± 0.12</td>
<td>5.35 ± 0.20</td>
<td>131.4 ± 37.1</td>
<td>0.53 ± 0.052</td>
</tr>
<tr>
<td>Control</td>
<td>1.08 ± 0.15</td>
<td>5.46 ± 0.23</td>
<td>116.5 ± 21.5</td>
<td>0.44 ± 0.05</td>
</tr>
</tbody>
</table>

1 Mean ±SEM; n = 19. Means were compared using the Student’s t-tests for correlated data.

2 Significantly different from the control trial, P = 0.039.

4.3.4 Plasma concentrations in the postprandial state

Changes in plasma volume during the observation periods were small (exercise, 1.3±1.6%; control, 4.2±2.0%) and did not differ significantly between trials. Thus, plasma concentrations were not adjusted for changes in plasma volume. Plasma triacylglycerol responses to the test meals are shown in Figure 4.2 and the total and incremental areas under the triacylglycerol concentration versus time curve are given in Table 4.2. The total area under the plasma triacylglycerol versus time curve was 18% lower on the exercise trial compared with the control trial.
Figure 4.2 Mean (± SEM) fasting and postprandial plasma concentrations of triacylglycerol for exercise (O) and control (■) trials (n = 19); black rectangles indicate consumption of the test meals. Data were analysed using two-way ANOVA with repeated measures. Main effect of trial (P = 0.009), main effect of time (P < 0.0005), trial × time interaction (P = 0.043).
Table 4.2 Seven-hour areas under the plasma concentration *versus* time curve for triacylglycerol (TAG), glucose, insulin and non-esterified fatty acids (NEFA) on the exercise and control trials.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Total TAG</th>
<th>Incremental TAG</th>
<th>Glucose mmol·7h/L</th>
<th>Insulin pmol·7h/L</th>
<th>NEFA mmol·7h/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmol·7h/L</td>
<td>mmol·7h/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise</td>
<td>10.02 ± 1.24²</td>
<td>3.24 ± 0.63</td>
<td>37.50 ± 1.02</td>
<td>2481.7 ± 465.0</td>
<td>1.73 ± 0.12³</td>
</tr>
<tr>
<td>Control</td>
<td>12.15 ± 1.62</td>
<td>4.83 ± 0.99</td>
<td>36.75 ± 1.08</td>
<td>2758.4 ± 475.1</td>
<td>1.48 ± 0.12</td>
</tr>
</tbody>
</table>

¹ Mean ±SEM; *n* = 19. Means were compared using the Student’s *t*-tests for correlated data.

² Significantly different from the control trial, *P* = 0.009.

³ Significantly different from the control trial, *P* = 0.014.

Plasma glucose, insulin and NEFA responses to the test meals are shown in Figures 4.3, 4.4 and 4.5, respectively. The total area under the concentration *versus* time curves for these parameters is given in Table 4.2. Plasma glucose concentrations did not differ between trials. The area under the curve value for plasma insulin tended to be lower on the exercise trial than the control trial but this difference was not significant (*P* = 0.07). The area under the curve value for plasma NEFA concentrations was higher on the exercise trial compared with the control trial.
Figure 4.3 Mean (± SEM) fasting and postprandial plasma concentrations of glucose for exercise (○) and control (■) trials (n = 19); the black rectangles indicate consumption of the test meals. Data were analysed using two-way ANOVA with repeated measures. Main effect of time (P < 0.0005).
Figure 4.4 Mean (± SEM) fasting and postprandial plasma concentrations of insulin for exercise (○) and control (■) trials (n = 19); the black rectangles indicate consumption of the test meals. Data were analysed using two-way ANOVA with repeated measures. Main effect of time (P < 0.0005).
Figure 4.5 Mean (± SEM) fasting and postprandial plasma concentrations of non-esterified fatty acids (NEFA) for exercise (○) and control (■) trials (n = 19); the black rectangles indicate consumption of the test meals. Data were analysed using two-way ANOVA with repeated measures. Main effects of trial (P = 0.014) and time (P < 0.0005).

4.4 Discussion

The present study demonstrates that by accumulating short bouts of running throughout the day postprandial triacylglycerol concentrations are lowered on the following day. Since postprandial lipaemia is a marker for cardiovascular disease risk the finding has important implications with respect to guidelines for physical activity and health.
Many studies have demonstrated that postprandial lipaemia is lowered following a single bout of aerobic exercise. The energy expended during exercise is an important determinant of the extent to which triacylglycerol is lowered (Gill and Hardman, 2003; Petitt and Cureton, 2003). To author’s knowledge, only three studies have examined the effects of accumulated/intermittent activity on postprandial lipaemia (Altena et al., 2004; Gill et al., 1998; Murphy et al., 2000). However, the present study was the first to examine the effects of accumulating short (<10 minute) bouts of activity on postprandial lipaemia.

Two mechanisms have been proposed to explain the reduction of postprandial triacylglycerol concentrations following exercise. One is increased muscle lipoprotein lipase activity, which is a major factor for the removal of triacylglycerol from the circulation (Seip and Semenkovich, 1998). Another is reduced secretion of hepatic VLDL (Gill et al., 2001a). It is impossible to tell which of these mechanisms predominates in the present study. The lower insulin concentrations during the exercise trial would suggest reduced inhibition of muscle lipoprotein lipase activity and therefore increased triacylglycerol clearance via the muscle. However, this would also indicate reduced uptake of insulin-mediated fatty acids into adipose tissue. Moreover, the elevated NEFA concentrations on the exercise trial could provide material for hepatic VLDL synthesis but this could be averted by increased fatty acid oxidation in the liver, leading to a reduced hepatic VLDL-triacylglycerol production.

Most studies examining postprandial triacylglycerol responses following exercise have employed a single test meal. In the present study, two test meals were consumed during a seven-hour period. The aim was to more accurately mimic a real life situation where
several meals are consumed throughout the day. The data demonstrate that the reduction in postprandial lipaemia with exercise is more marked following the second meal compared with the first meal. This is consistent with the findings of a previous study (Murphy et al., 2000). This attenuation could be protective against atherosclerosis since repeated daily disturbances of postprandial triacylglycerol concentration are related to the progression of atherosclerosis (Geluk et al., 2004).

The energy expended during accumulated exercise in the current study was high (4.2 MJ (1000 kcal)) as was the exercise intensity (70% of maximum oxygen uptake). An energy expenditure of 4.2 MJ (1000 kcal) was chosen because previous research indicates that this level of expenditure is effective in lowering postprandial lipaemia with continuous exercise (Petitt and Cureton, 2003). The author wanted to be sure that if the study did not find an influence of accumulated activity on postprandial lipaemia it was not because of insufficient energy expenditure. It is important to note, however, that this expenditure is up to five times higher than that which would occur following adherence to the minimal recommended guideline of 30 minutes of moderate-intensity exercise per day (Pate et al., 1995; U.K. Department of Health, 2004; U.S. Department of Health and Human Services, 1996). In fact some dispute that adherence to the minimum guidelines (both in terms of volume and intensity) would have the desired impact on health (Barinaga, 1997). The present study has advanced understanding of intermittent/accumulated activity by demonstrating that the accumulation of very short (<10 minute) bouts of activity can be effective in lowering postprandial lipaemia.

One final issue worthy of mention before concluding this discussion is the nature of the study participants. The present study elected to study lean, fit subjects because such a
group provided a convenient sample to test the hypothesis that accumulating very short bouts of activity would be effective in lowering postprandial lipaemia. It could be speculated that since triacylglycerol concentrations were low in this group that the potential for reductions in these variables was limited and greater reductions may be seen in those with hypertriacylglycerolaemia. Conversely, subjects with lower fitness and higher levels of body fat would have greater difficulty expending 4.2 MJ (1000 kcal) in accumulated activity and therefore changes in triacylglycerol may be less dramatic. This illustrates the need for further research employing sedentary individuals performing a volume and intensity of activity, which more closely resembles the current guidelines (Pate et al., 1995; U.K. Department of Health, 2004; U.S. Department of Health and Human Services, 1996).

In conclusion, these data show that performing multiple 6 minute bouts of running during a single day reduces postprandial plasma triacylglycerol concentrations on the following day in healthy young men. This study provides further support for the concept of accumulating exercise and suggests that a minimum exercise duration of 10-minutes may be unnecessary. However, the present study was an efficacy study employing high intensity and high volume exercise. Additional research will be required to determine if similar results can be obtained with short bouts of moderate intensity exercise, and with lower total daily energy expenditure accumulated from the short bouts. If these efficacy trials provide similar results, then effectiveness studies can be done to determine if sedentary free-living individuals can obtain similar benefits with exercise programmes that they can maintain.
CHAPTER 5

EXERCISE AND POSTPRANDIAL LIPAEMIA: EFFECT OF CONTINUOUS VERSUS INTERMITTENT ACTIVITY PATTERNS

5.1 Introduction

The study reported in the previous chapter demonstrated that accumulating a large volume (4.2 MJ = 1000 kcal) of exercise in short (6 minute) bouts performed throughout a day is effective in lowering postprandial triacylglycerol concentrations on the following day. The study suggests that a minimum duration of 10 minutes may be unnecessary for health benefits provided sufficient energy is expended, at least in the case of postprandial lipaemia. However, the study employed a high volume of exercise. Further research is needed to determine whether accumulating short bouts of exercise with lower total energy expenditure is effective for lowering postprandial triacylglycerol concentrations. In addition, the study reported in the previous chapter did not include a continuous trial of matched total energy expenditure to the accumulated trial. Therefore, whether the benefit obtained through multiple short bouts of exercise is equal to one longer bout of exercise remains to be determined.

Therefore, the purpose of the present study was to compare postprandial triacylglycerol concentrations in a group of young healthy males following either 30 minutes of continuous exercise or 30 minutes of accumulated activity i.e. ten, three-minute bouts performed throughout a single day. An exercise volume of 30 minutes was chosen since this is consistent with current guidelines regarding physical activity and health (Pate et al., 1995; U.K. Department of Health, 2004; U.S. Department of Health and Human Services, 1996). Exercise durations of three minute were selected for the
accumulated activity trial so that bouts could be performed throughout the day with a
30 minute rest interval between each bout.

5.2 Methods

5.2.1 Subjects
Ten healthy males (aged 21 to 32 years) volunteered to participate in this study. All
subjects were recreationally active and had been weight stable (± 2.5 kg) for at least
three months before the study. To minimise risks, subjects were only recruited if they
met the following criteria: were non-smoking, were free of known cardiovascular
disease or abnormalities, were not taking any medication known to influence lipid or
carbohydrate metabolism, had resting arterial blood pressure <140/90 mm/Hg, and had
a BMI <35 kg/m². Mean (± SEM) values for the subjects’ age, height, weight, BMI,
waist circumference, percentage body fat and maximum oxygen uptake were: 25.0 ±
1.3 y, 177.4 ± 1.4 cm, 80.2 ± 4.8 kg, 25.4 ± 1.2 kg/m², 87.2 ± 3.5 cm, 9.4 ± 0.7 % and
56.3 ± 1.8 mL/kg/min, respectively. The University’s Ethical Advisory Committee
approved the study and written informed consent was obtained from all subjects prior
to their participation in the study.

5.2.2 Preliminary testing
Anthropometric measurements were made as described in section 3.2. Subjects then
performed submaximal (section 3.6.1) and maximal (section 3.6.2) incremental
exercise tests to establish the running speed required to elicit 70% of maximum oxygen
uptake in the main trials.
5.2.3 Main trials

Each subject underwent three, 2-day trials: an accumulated exercise trial, a continuous exercise trial, and a control trial. Two-day trials were used (control/exercise day 1, postprandial testing day 2) because skeletal muscle lipoprotein lipase activity is thought to peak >8 hours after exercise (Seip et al., 1997) and this enzyme facilitates the removal of triacylglycerol from the blood (Seip and Semenkovich, 1998). There was a seven-day gap between each trial and trials were performed in a randomised design (for the schematic representation of the study protocol, see Figure 5.1).
Accumulated exercise trial: subjects performed ten, 3-minute runs (at 70% of maximal oxygen uptake) throughout the day with 30 minute rest intervals between bouts.

Continuous exercise trial: subjects performed one, 30-minute run (at 70% of maximal oxygen uptake) in the afternoon (15:00-15:30).

Control trial: subjects rested in the laboratory throughout the day.

Consumption of packed lunch

**Figure 5.1** Schematic representation of the study protocol
Day 1. On the first day of each trial, subjects reported to the laboratory at 09:00 having eaten breakfast. For the accumulated exercise trial subjects performed ten, three-minute bouts of treadmill running throughout the day. A 30-minute rest interval followed each bout. On the continuous exercise trial subjects performed one 30 minute treadmill run in the afternoon. For both of these trials, the exercise was completed at 15:30 so that the time interval between the cessation of exercise and the consumption of the first test meal (on the following day) was the same as in the accumulated trial (i.e. 17 hours). The exercise intensity for the treadmill running was 70% of maximum oxygen uptake as determined by the preliminary exercise tests. The expired air samples - collected into Douglas bags during the last minute of each bout of exercise throughout day 1 on the accumulated exercise trial and collected at 9-10, 19-20 and 29-30 minutes during the 30-minute exercise session on day 1 of the continuous exercise trial - were used to determine the actual energy expenditure during exercise (section 3.7). Heart rate was monitored during each bout of exercise using short-range telemetry (section 3.3). Ratings of perceived exertion were assessed periodically during each bout of exercise using the Borg scale (Borg, 1973) (section 3.4). On the control trial subjects rested throughout the day in the laboratory. During each trial, subjects consumed a packed lunch midway through the day (12:00-13:00). Subjects left the laboratory at approximately 16:00 and they were instructed to consume an early evening meal and rest for the remainder of the evening.

Day 2. On the second day of each trial subjects reported to the laboratory at 08:00 after a 10 hour overnight fast (no food or drink except water). Subjects sat in a semi-supine position on a bed for 10 minutes following arrival at the laboratory. A cannula (Venflon, Becton Dickinson, Helsingborg, Sweden) was then inserted into an antecubital vein
and a baseline blood sample was collected (section 3.10). Subjects then consumed a standardised test meal for breakfast (section 3.9). A clock was started when subjects began eating and they were required to rest (reading, watching television) in the laboratory for seven hours following the initiation of breakfast. A second test meal (identical to the first) was consumed three hours after the initiation of the first meal. Venous blood samples were collected at hourly intervals throughout the day for the measurement of triacylglycerol, glucose, insulin, NEFA and 3-hydroxybutyrate (section 3.11). Additional samples were collected at 0.5, 0.75, 3.5 and 3.75 hours for measurement of glucose and insulin.

5.2.4 Standardisation of diet and exercise

Subjects weighed and recorded all food and drink consumed for two days prior to any of the main trials. Subjects then consumed identical amounts of the same food and drink prior to each of the main trials. Thus, meals were standardised across trials including the evening meal on day 1. Subjects refrained from drinking alcohol during this time. In addition, subjects were asked to remain inactive on the day before day 1 of each trial and throughout the main trials (other than the exercise performed as part of the experiment). Food diaries were analysed using computerised software (Comp-EAT Version 5.0, Nutrition Systems, London, United Kingdom) to determine energy intake and macronutrient content.

5.2.5 The test meals

Full details of the tests meal are given in section 3.9. The average macronutrient content of each test meal was $55.5 \pm 3.2$ g fat, $72.5 \pm 4.2$ g carbohydrate, and $25.0 \pm 1.5$ g protein which provided $3.70 \pm 0.22$ MJ (769 ± 52 kcal) of energy (56% fat, 33%
carbohydrate and 11% protein). Subjects were asked to consume each meal within 20 minutes. The time taken to consume each meal was recorded and replicated in subsequent trials. Average time taken to consume the first meal (breakfast) was $12.0 \pm 1.0$ minutes. Average time taken to consume the second meal (lunch) was $14.2 \pm 1.3$ minutes. None of the subjects reported nausea or any gastrointestinal discomfort during or after meals. Subjects consumed water ad libitum during the first trial and the volume ingested was replicated in subsequent trials.

5.2.6 Blood sample analysis

Blood samples were analysed for triacylglycerol, glucose, 3-hydroxybutyrate, NEFA and insulin. Full details of the blood sample analysis are given in section 3.11. Intra-assay coefficients of variation were 0.5% for triacylglycerol, 0.8% for glucose, 5.1% for 3-hydroxybutyrate, 1.7% for NEFA, and 7.4% for insulin. Homeostatic model assessment (HOMA) was used to estimate whole-body insulin sensitivity, calculated as fasting insulin (microunits per milliliter) $\times$ fasting glucose (millimole per liter)/22.5 (Matthews et al., 1985). Hemoglobin concentration and hematocrit were measured at baseline and the end of observation period for estimating changes in plasma volume (Dill and Costill, 1974) (section 3.11.1).

5.2.7 Statistical analysis

Data were analysed using the SPSS software version 12.0 for Windows (SPSS Inc, Chicago, U.S.A.). The Shapiro-Wilk test was used to check for normality. Area under the curve values for plasma concentrations over time were calculated using the trapezium rule. Student's $t$-tests for correlated data were used to compare physiological responses between exercise trials (energy expenditure, RPE, respiratory exchange ratio,
oxygen consumption and heart rate). One-way ANOVA was used to examine differences between the three trials for fasting plasma concentrations, area under the curve values and percentage change in plasma volume. Two-way ANOVA was used to examine differences between the three trials over time for plasma constituents. Where significant interactions were found, post-hoc multiple comparisons were made using the Bonferroni method. Statistical significance was accepted at the 5% level. Results are presented as mean ± SEM.

5.3 Results

5.3.1 Responses to treadmill running

Exercise data are shown in Table 5.1. There were no significant differences between exercise trials in the estimated gross energy expenditure, relative exercise intensity, RPE or respiratory exchange ratio. Mean values for heart rate were significantly higher on the continuous exercise trial compared with the accumulated exercise trial. For comparison, average heart rate on day 1 of the control trial (mean of ten heart rate measurements collected at identical time points to those in the accumulated exercise trial) was 63 ± 2 beats/min.
Table 5.1 Estimated energy expenditure, percentage of maximal oxygen uptake (%VO₂max), heart rate, ratings of perceived exertion (RPE) and respiratory exchange ratio during the accumulated and continuous exercise trials.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Energy expenditure (MJ/30 min)</th>
<th>%VO₂max (mL/kg/min)</th>
<th>Heart rate (beats/min)</th>
<th>RPE</th>
<th>Respiratory exchange ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accumulated</td>
<td>1.96 ± 0.09</td>
<td>69.6 ± 1.0</td>
<td>153 ± 3</td>
<td>11 ± 0</td>
<td>0.97 ± 0.01</td>
</tr>
<tr>
<td>exercise²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous</td>
<td>2.01 ± 0.11</td>
<td>71.1 ± 2.3</td>
<td>167 ± 3</td>
<td>12 ± 1</td>
<td>0.99 ± 0.01</td>
</tr>
<tr>
<td>exercise³</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Mean ± SEM; n = 10. Means were compared using Student's t-test for correlated data.
² calculated from data collected during min 2-3 of all ten exercise bouts.
³ calculated from data collected at 9-10, 19-20 and 29-30 min of exercise.
⁴ Significantly different from the accumulated exercise trial, P < 0.0005.

5.3.2 Dietary data

Average energy intakes for the pre-trial day and for day 1 of the trials were 10.83 ± 1.12 MJ (2589 ± 279 kcal) and 10.94 ± 1.12 MJ (2615 ± 269 kcal), respectively. Average dietary intake of fat, carbohydrate and protein was 101.2 ± 15.7 g, 317.1 ± 39.5 g and 124.2 ± 20.8 g respectively, for the pre-trial day, and 87.4 ± 9.5 g, 372.6 ± 151.1 g and 108.4 ± 13.5 g respectively, for day 1.
5.3.3 Plasma concentrations in the fasted state

Fasting plasma concentrations prior to the test meals on day 2 of each trial are shown in Table 5.2. One-way ANOVA revealed a main effect of trial for fasting plasma triacylglycerol, glucose, and 3-hydroxybutyrate between trials. Post-hoc tests showed that fasting plasma glucose concentration was lower on the accumulated exercise trial than the control trial. Although post-hoc tests did not reveal a significant difference in fasting plasma triacylglycerol concentrations between trials, plasma triacylglycerol concentrations tended to be lower on both exercise trials than on the control trial (accumulated exercise versus control, \( P = 0.180 \); continuous exercise versus control, \( P = 0.096 \)). Similarly, post-hoc tests did not reveal any significant between trial differences for fasting plasma 3-hydroxybutyrate concentrations but values tended to be higher in both exercise trials (accumulated exercise versus control, \( P = 0.057 \); continuous exercise versus control, \( P = 0.193 \)) than on the control trial. There was no difference in fasting insulin concentrations between trials. Moreover, there was no significant difference in the HOMA insulin sensitivity index between trials (accumulated exercise trial, \( 4.7 \pm 0.7 \); continuous exercise trial, \( 4.6 \pm 0.5 \); control trial, \( 5.4 \pm 1.2 \)).
Table 5.2 Fasting plasma concentrations of triacylglycerol (TAG), glucose, insulin, non-esterified fatty acids (NEFA) and 3-hydroxybutyrate (3-OHB) for the accumulated exercise, continuous exercise and control trials

<table>
<thead>
<tr>
<th>Trial</th>
<th>TAG (mmol/L)</th>
<th>Glucose (mmol/L)</th>
<th>Insulin (pmol/L)</th>
<th>NEFA (mmol/L)</th>
<th>3-OHB (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accumulated exercise</td>
<td>1.14 ± 0.19</td>
<td>5.05 ± 0.08²</td>
<td>143.3 ± 19.8</td>
<td>0.38 ± 0.05</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>Continuous exercise</td>
<td>1.10 ± 0.20</td>
<td>5.09 ± 0.08</td>
<td>141.3 ± 14.4</td>
<td>0.42 ± 0.04</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td>Control</td>
<td>1.35 ± 0.28</td>
<td>5.21 ± 0.97</td>
<td>158.5 ± 32.0</td>
<td>0.36 ± 0.04</td>
<td>0.02 ± 0.01</td>
</tr>
</tbody>
</table>

¹ Mean ±SEM; n = 10. Means were compared using one-way ANOVA for the main effect of trial followed by a Bonferroni multiple comparisons test.

² Main effect of trial, P < 0.04.

³ Significantly different from the control trial, P = 0.04.

5.3.4 Plasma concentrations in the postprandial state

Changes in plasma volume during the observation periods were small and did not differ significantly between trials (accumulated exercise, -0.2±2.3%; continuous exercise, -0.3±1.9%; control, -1.5±1.5%). Thus, plasma concentrations were not adjusted for changes in plasma volume.
Plasma triacylglycerol responses to the test meals are shown in Figure 5.2. Two-way ANOVA revealed significant main effects of trial and time and a significant interaction. Plasma triacylglycerol responses were lower on the exercise trials compared with the control trial (post-hoc tests, accumulated exercise versus control, $P = 0.019$; continuous exercise versus control, $P = 0.019$) with little difference between the accumulated and continuous exercise trials. Total and incremental areas under the triacylglycerol concentration versus time curve are given in Table 5.3. There was a main effect of trial for both of these variables. The total area under the plasma triacylglycerol versus time curve was 22% and 24% lower on the accumulated and continuous exercise trials respectively, compared with the control trial. The incremental area under the plasma triacylglycerol versus time curve was 31% and 32% lower on the accumulated and continuous exercise trials respectively, compared with the control trial. There were no significant differences between the exercise trials for plasma triacylglycerol concentration.
Figure 5.2 Mean (± SEM) fasting and postprandial plasma concentrations of triacylglycerol for accumulated exercise (●), continuous exercise (△) and control (■) trials (n = 10); black rectangles indicate consumption of the test meals. Data were analysed using two-way ANOVA followed by the Bonferroni multiple comparisons test. Main effect of trial (P = 0.001), main effect of time (P < 0.0005), trial × time interaction (P = 0.046).
Table 5.3 Seven-hour areas under the plasma concentration versus time curve for total triacylglycerol (TAG) and incremental TAG (adjusted for fasting value) on the accumulated exercise, continuous exercise and control trials

<table>
<thead>
<tr>
<th>Trial</th>
<th>Total TAG(^2) (mmol·7h/L)</th>
<th>Incremental TAG(^2) (mmol·7h/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accumulated exercise</td>
<td>13.25 ± 2.24(^3)</td>
<td>5.18 ± 1.05(^3)</td>
</tr>
<tr>
<td>Continuous exercise</td>
<td>12.82 ± 2.26(^3)</td>
<td>5.11 ± 0.91(^3)</td>
</tr>
<tr>
<td>Control</td>
<td>16.96 ± 3.09</td>
<td>7.49 ± 1.34</td>
</tr>
</tbody>
</table>

\(^1\) Mean ± SEM; \(n = 10.\) Means were compared using one-way analysis of variance followed by a Bonferroni multiple comparisons test.

\(^2\) Main effect of trial, \(P = 0.001.\)

\(^3\) Significantly different from the control trial, \(P \leq 0.02.\)

Plasma glucose, insulin, NEFA and 3-hydroxybutyrate responses to the test meals are shown in Figures 5.3, 5.4, 5.5 and 5.6, respectively. There was a main effect of time for both glucose and insulin. For insulin there was also a main effect of trial. Post-hoc tests revealed that plasma insulin concentration was lower on the accumulated exercise trial compared with the control trial \((P = 0.047).\) There was no difference in plasma insulin concentration between the continuous exercise and control trials or between the accumulated exercise and continuous exercise trials. No differences were observed between trials for plasma concentrations of NEFA and 3-hydroxybutyrate.
Figure 5.3 Mean (± SEM) fasting and postprandial plasma concentrations of glucose for accumulated exercise (●), continuous exercise (△) and control (■) trials (n = 10); the black rectangles indicate consumption of the test meals. Data were analysed using two-way ANOVA followed by the Bonferroni multiple comparisons test. Main effect of time (P < 0.0005).
Figure 5.4 Mean (± SEM) fasting and postprandial plasma concentrations of insulin for accumulated exercise (●), continuous exercise (△) and control (■) trials (n = 10); the black rectangles indicate consumption of the test meals. Data were analysed using two-way ANOVA followed by the Bonferroni multiple comparisons test. Main effect of trial (P = 0.038), main effect of time (P < 0.0005).
Figure 5.5 Mean (± SEM) fasting and postprandial plasma concentrations of non-esterified fatty acids (NEFA) for accumulated exercise (●), continuous exercise (△) and control (■) trials (n = 10); the black rectangles indicate consumption of the test meals. Data were analysed using two-way ANOVA with repeated measures followed by the Bonferroni multiple comparisons test. Main effect of time (P < 0.0005).
Figure 5.6 Mean (± SEM) fasting and postprandial plasma concentrations of 3-hydroxybutyrate for accumulated exercise (●), continuous exercise (△) and control (■) trials (n = 10); the black rectangles indicate consumption of the test meals. Data were analysed using two-way ANOVA with repeated measures followed by the Bonferroni multiple comparisons test. Main effect of time (P = 0.013).

5.4 Discussion

The main finding of the present study is that postprandial triacylglycerol concentration is reduced to a similar extent when ten, three-minute bouts of running are performed during the course of a day in comparison to one continuous 30 minute bout of exercise. This finding suggests that health benefits may accrue from the accumulation of very short bouts of physical activity. Therefore, the current recommendation (Pate et al., 1995; U.K. Department of Health, 2004; U.S. Department of Health and Human Services, 1996) that each individual exercise bout should last at least 10-minutes may
be unnecessary – at least in the case of postprandial triacylglycerol concentrations. Completing many short bouts of physical activity throughout the day may be easier and more appealing for many individuals compared to a pattern involving longer activity bouts. Moreover, evidence suggests that for many people who manage to perform 30 minutes of physical activity per day, this is achieved by accumulating several short (<10 minute) bouts (Jakicic et al., 1995).

Many studies have demonstrated that postprandial triacylglycerol concentration is lowered following a single bout of aerobic exercise. The energy expended during exercise appears to be an important determinant of the extent to which triacylglycerol is lowered (Gill and Hardman, 2003; Petitt and Cureton, 2003). Three previous studies have examined the effects of accumulated/intermittent activity on postprandial triacylglycerol concentration (Altena et al., 2004; Gill et al., 1998; Murphy et al., 2000). The minimum duration of any one activity bout in these studies was 10 minutes. In addition, the study reported in Chapter 4 demonstrated that accumulating 6 minute bouts of exercise is effective in lowering postprandial triacylglycerol concentrations. To author’s knowledge, the present study is the first to examine the effects of accumulating very short (3 minute) bouts of activity on postprandial lipaemia. Taken together these findings suggest that the duration of individual exercise bouts is unimportant for lowering triacylglycerol providing that sufficient energy is expended throughout the day.

Two mechanisms have been proposed to explain reductions in postprandial triacylglycerol concentrations following exercise. One is increased activity of the enzyme lipoprotein lipase located in the capillaries supplying skeletal muscle. This
would facilitate the clearance of triacylglycerol from plasma into muscle to replace the intramuscular triacylglycerol oxidised during exercise (Seip and Semenkovich, 1998). The other mechanism is a reduced synthesis and secretion of VLDL-triacylglycerol from the liver (Gill et al., 2001a). It is impossible to tell which of these mechanisms was operating in the present study. Non-esterified fatty acid concentrations did not differ between trials in the present study suggesting that substrate delivery to the liver for triacylglycerol synthesis and secretion in VLDLs was not different between trials. However, fasting plasma 3-hydroxybutyrate concentrations were elevated on the exercise trials and this indicates that fatty acid oxidation in the liver was elevated, thus reducing the availability of triacylglycerol for incorporation into VLDL. In contrast, the reduced plasma insulin concentrations in the accumulated exercise trial (and the tendency for reduced concentrations in the continuous exercise trial) suggest reduced insulin mediated inhibition of skeletal muscle lipoprotein lipase activity and therefore enhanced triacylglycerol clearance at this site (Seip and Semenkovich, 1998).

The exercise intensity employed in the present study was high i.e. 70% of maximum oxygen uptake. This high exercise intensity necessitated a high rate of energy expenditure i.e. 67 kJ/min (16 kcal/min). This ensured that the total amount of energy expended in exercise was high i.e. 2 MJ (476 kcal) during 30 minutes of exercise. This is more than twice the expenditure that would be attained by the average adult completing 30 minutes of moderate intensity physical activity i.e. 200 kcal according to Pate et al (1995). Further research is required to ascertain whether 30-minutes of moderate intensity physical activity (50 to 60% of maximum oxygen uptake) accumulated in very short bouts is effective in lowering postprandial triacylglycerol concentration. It is worth noting, however, that for sedentary older and slightly
The participants in the present study were young, regularly active healthy males. Therefore, the findings cannot be generalised to young, sedentary adults or to middle-aged and older adults. However, it is possible that exercise induced reductions in postprandial lipaemia would be greater in these groups since perturbations in triacylglycerol concentrations following meals are more likely to be exaggerated in such individuals. Furthermore, Murphy et al (2000) have demonstrated that postprandial triacylglycerol concentrations are reduced in postmenopausal sedentary women following 30-minutes of brisk walking at an intensity equivalent to 60% of maximal oxygen uptake. In this study exercise was undertaken in either one continuous session or accumulated in three, ten-minute bouts. Thus, a question which needs addressing in future studies is whether adherence to the minimal recommended volume of exercise (0.84 MJ = 200 kcal) through multiple short (< 10 minute) bouts of moderate intensity physical activity is effective in lowering postprandial triacylglycerol concentrations in subjects of varying age and varying habitual activity patterns.

The test meals employed in this study were high in fat and low in carbohydrate (56% fat, 33% carbohydrate and 11% protein). The macronutrient content of these test meals does not reflect that in a typical Western diet. However, if the fat content of the meals was replaced with carbohydrate this could exacerbate the triacylglycerol response further due to carbohydrate-induced hypertriacylglycerolaemia (Parks and Hellerstein, 2000). Further research examining the effects of accumulated physical activity on
postprandial triacylglycerol concentrations following meals typically consumed by Western populations would be useful.

Two other findings from this study require explanation. Firstly, the present study observed a higher mean heart rate during the continuous exercise trial compared with the accumulated exercise trial. This difference may be due to cardiovascular drift, which is known to occur as exercise progresses (Coyle and Gonzalez-Alonso, 2001). Secondly, fasting blood glucose was lower on day 2 of the accumulated exercise trial compared with day 2 of the control trial. A trend was also noted for lower fasting blood glucose concentration on day 2 of the continuous exercise trial compared with day 2 of the control trial. It is possible that this was due to reduced liver glycogen concentration following exercise and hence reduced hepatic glucose output.

In conclusion, the results of present study demonstrate that in young healthy males, 30 minutes of running accumulated in ten, three-minute bouts is equally as effective in lowering postprandial triacylglycerol concentrations as 30 minutes of activity performed in one continuous bout. These findings suggest that – as far as exercise guidelines for health are concerned – it may be unnecessary to stipulate that physical activity be performed in bouts of 10 minutes or longer. Further research is required to determine if these findings can be generalised to the public at large.
CHAPTER 6
EFFECT OF CONTINUOUS VERSUS INTERMITTENT
BRISK WALKING ON POSTPRANDIAL
PLASMA TRIACYLGLYCEROL CONCENTRATIONS

6.1 Introduction
The study reported in Chapter 4 demonstrated that accumulating short (6 minute) bouts of running throughout the day is effective in reducing postprandial triacylglycerol concentrations on the following day. However, a large volume (4.2 MJ = 1000 kcal) of exercise was performed by young men in this study. Such a large volume of exercise would not be achievable by many individuals.

Thus, in the study reported in Chapter 5 exercise volume (2.0 MJ = 476 kcal) was reduced by more than half and this study demonstrated that accumulating 30 minutes of running in very short (3 minute) bouts of running is equally effective in reducing postprandial triacylglycerol concentrations on the following day as one continuous 30 minute run. In this study, the exercise duration was consistent with guidelines for health (Pate et al., 1995; U.K. Department of Health, 2004; U.S. Department of Health and Human Services, 1996). However, the total energy expenditure was still high (2.0 MJ = 476 kcal) compared with the minimum expenditure (200 kcal) recommended by current physical activity guidelines (Pate et al., 1995). In addition, the exercise intensity (70% of maximal oxygen uptake) is still not applicable for many middle-aged people and the mode of exercise was running which is associated with a higher rate of injury compared with walking (Morris and Hardman, 1997). Thus, whether similar benefit can be obtained, as in previous studies (Chapters 4 and 5) with the
accumulation of 30 minutes of activity of reduced intensity and energy expenditure remains to be determined.

Therefore, the purpose of the present study was to compare plasma triacylglycerol responses to test meals when 30 minutes of brisk walking was performed throughout the day in ten, three-minute bouts or one, 30 minute bout. The study protocol was identical to that used in the previous study (Chapter 5) except that walking was the mode of exercise rather than running. Walking was chosen for this study since it is the most popular form of activity and can be incorporated into daily activity routines with little injury risk (Morris and Hardman, 1997).

6.2 Methods

6.2.1 Subjects

Fifteen healthy males (aged 18 to 28 years) volunteered to participate in this study. All subjects were recreationally active and had been weight stable (± 2.5 kg) for at least three months before the study. To minimise risks, subjects were only recruited if they met the following criteria: were non-smoking, were free of known cardiovascular disease or abnormalities, were not taking any medication known to influence lipid or carbohydrate metabolism, had resting arterial blood pressure <140/90 mm Hg, and had a BMI <35 kg/m². Mean (± SEM) values for the subjects’ age, height, weight, BMI, waist circumference, percentage body fat and maximum oxygen uptake were: 23.4 ± 0.8 y, 178.6 ± 1.3 cm, 74.9 ± 2.3 kg, 23.4 ± 0.6 kg/m², 80.8 ± 2.1 cm, 11.2 ± 0.9 % and 56.3 ± 2.1 mL/kg/min, respectively. The University’s Ethical Advisory Committee approved the study and written informed consent was obtained from all subjects prior to their participation in the study.
6.2.2 Preliminary testing

Anthropometric measurements were made as described in section 3.2. Thereafter, walking speed on a treadmill at a self-selected brisk pace was assessed (section 3.6.3). Subjects then performed submaximal (section 3.6.1) and maximal (section 3.6.2) incremental exercise tests. The data from the submaximal and maximal exercise tests were used to determine the relative exercise intensity adopted by each subject at their self-selected brisk walking pace.

6.2.3 Main trials

Each subject underwent three, 2-day trials: an accumulated exercise trial, a continuous exercise trial, and a control trial. Two-day trials were used (control/exercise day 1, postprandial testing day 2) because skeletal muscle lipoprotein lipase activity is thought to peak >8 hours after exercise (Seip et al., 1997) and this enzyme facilitates the removal of triacylglycerol from the blood (Seip and Semenkovich, 1998). There was a seven-day gap between each trial and trials were performed in a randomised design (for the schematic representation of the study protocol, see Figure 6.1).
Accumulated exercise trial: subjects performed ten, 3-minute bouts of walking (at a self-selected brisk pace) throughout the day with 30 minute rest intervals between bouts.

Continuous exercise trial: subjects performed one, 30-minute bout of walking (at a self-selected brisk pace) in the afternoon (15:00-15:30).

Control trial: subjects rested in the laboratory throughout the day.

Consumption of packed lunch

Figure 6.1 Schematic representation of the study protocol
Day 1. On the first day of each trial, subjects reported to the laboratory at 09:00 having eaten breakfast. For the accumulated exercise trial subjects performed ten, three-minute bouts of treadmill brisk walking throughout the day. A 30-minute rest interval followed each bout. On the continuous exercise trial subjects performed one 30 minute treadmill brisk walk in the afternoon. For both of these trials, the exercise was completed at 15:30 so that the time interval between the cessation of exercise and the consumption of the first test meal (on the following day) was the same (i.e. 17 hours). Walking speed was set at self-selected pace as determined by the preliminary visit. The expired air samples - collected into Douglas bags during the last minute of each bout of exercise throughout day 1 on the accumulated exercise trial and collected at 9-10, 19-20 and 29-30 minutes during the 30-minute exercise session on day 1 of the continuous exercise trial - were used to determine the actual energy expenditure during exercise (section 3.7). Heart rate was monitored during each bout of exercise using short-range telemetry (section 3.3). Ratings of perceived exertion were assessed periodically during each bout of exercise using the Borg scale (Borg, 1973) (section 3.4). On the control trial subjects rested throughout the day in the laboratory. During each trial, subjects consumed a packed lunch midway through the day (12:00-13:00). Subjects left the laboratory at approximately 16:00 and they were instructed to consume an early evening meal and rest for the remainder of the evening.

Day 2. On the second day of each trial subjects reported to the laboratory at 08:00 after a 10 hour overnight fast (no food or drink except water). Subjects sat in a semi-supine position on a bed for 10 minutes following arrival at the laboratory. A cannula (Venflon, Becton Dickinson, Helsingborg, Sweden) was then inserted into an antecubital vein and a baseline blood sample was collected (section 3.10). Subjects then consumed a
standardised test meal for breakfast (section 3.9). A clock was started when subjects began eating and they were required to rest (reading, watching television) in the laboratory for seven hours following the initiation of breakfast. A second test meal (identical to the first) was consumed three hours after the initiation of the first meal. Venous blood samples were collected at hourly intervals throughout the day for the measurement of triacylglycerol, glucose and insulin (section 3.11). Additional samples were collected at 0.5, 0.75, 3.5 and 3.75 hours for measurement of glucose and insulin.

6.2.4 Standardisation of diet and exercise

Subjects weighed and recorded all food and drink consumed for two days prior to any of the main trials. Subjects then consumed identical amounts of the same food and drink prior to each of the main trials. Thus, meals were standardised across trials including the evening meal on day 1. Subjects refrained from drinking alcohol during this time. In addition, subjects were asked to remain inactive on the day before day 1 of each trial and throughout the main trials (other than the exercise performed as part of the experiment). Food diaries were analysed using computerised software (Comp-EAT Version 5.0, Nutrition Systems, London, United Kingdom) to determine energy intake and macronutrient content.

6.2.5 The test meals

Full details of the tests meal are given in section 3.9. The average macronutrient content of each test meal was 51.8 ± 1.5 g fat, 67.5 ± 2.0 g carbohydrate, and 23.3 ± 0.7 g protein which provided 3.45 ± 0.10 MJ (825 ± 24 kcal) energy (56% fat, 33% carbohydrate and 11% protein). Subjects were asked to consume each meal within 20 minutes. The time taken to consume each meal was recorded and replicated in
subsequent trials. Average time taken to consume the first meal (breakfast) was 10.1 ± 0.8 minutes. Average time taken to consume the second meal (lunch) was 11.0 ± 1.0 minutes. None of the subjects reported nausea or any gastrointestinal discomfort during or after meals. Subjects consumed water *ad libitum* during the first trial and the volume ingested was replicated in subsequent trials.

6.2.6 Blood sample analysis

Blood samples were analysed for triacylglycerol, glucose and insulin. Full details of the blood sample analysis are given in section 3.11. Intra-assay coefficients of variation were 0.5% for triacylglycerol, 1.8% for glucose, and 7.4% for insulin. Hemoglobin concentration and hematocrit were measured at baseline and the end of observation period for estimating changes in plasma volume (Dill and Costill, 1974) (section 3.11.1).

6.2.7 Statistical analysis

Data were analysed using the SPSS software version 12.0 for Windows (SPSS Inc, Chicago, U.S.A.). The Shapiro-Wilk test was used to check for normality. Area under the curve values for plasma concentrations over time were calculated using the trapezium rule. Student’s *t*-tests for correlated data were used to compare physiological responses between exercise trials (energy expenditure, RPE, respiratory exchange ratio, oxygen consumption and heart rate). One-way ANOVA was used to examine differences between the three trials for fasting plasma concentrations, area under the curve values and percentage change in plasma volume. Two-way ANOVA was used to examine differences between the three trials over time for plasma constituents. Where significant interactions were found, post-hoc multiple comparisons were made using
the Bonferroni method. Statistical significance was accepted at the 5% level. Results are presented as mean ± SEM.

6.3 Results

6.3.1 Responses to treadmill walking

Exercise data are shown in Table 6.1. There were no significant differences between exercise trials in the estimated gross energy expenditure, relative exercise intensity, oxygen uptake, metabolic equivalent (MET) values or respiratory exchange ratio. Mean values for heart rate and RPE were significantly higher on the continuous trial compared with the accumulated trial. Average heart rate on day 1 of the control trial (mean of ten heart rate measurements collected at identical time points to those in the accumulated trial) was 64 ± 2 beats/min.
Table 6.1 Estimated energy expenditure, percentage of maximal oxygen uptake, oxygen uptake, metabolic equivalent (MET) values, heart rate, ratings of perceived exertion (RPE) and respiratory exchange ratio during the accumulated and continuous exercise trials.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Energy Expenditure (MJ/30 min)</th>
<th>% maximal oxygen uptake (mL/kg/min)</th>
<th>Oxygen uptake (mL/kg/min)</th>
<th>MET values</th>
<th>Heart rate (beats/min)</th>
<th>RPE</th>
<th>Respiratory exchange ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accumulated exercise²</td>
<td>1.10 ± 0.04</td>
<td>41.4 ± 1.8</td>
<td>23.4 ± 0.7</td>
<td>6.7 ± 0.2</td>
<td>122 ± 3</td>
<td>9 ± 0</td>
<td>0.94 ± 0.02</td>
</tr>
<tr>
<td>Continuous exercise³</td>
<td>1.10 ± 0.11</td>
<td>42.4 ± 1.8</td>
<td>23.7 ± 0.7</td>
<td>6.8 ± 0.2</td>
<td>131 ± 4⁴</td>
<td>10 ± 0</td>
<td>0.97 ± 0.01</td>
</tr>
</tbody>
</table>

¹ Mean ± SEM; n = 15. Means were compared using Student’s t-test for correlated data.

² calculated from data collected during min 2-3 of all ten exercise bouts

³ calculated from data collected at 9-10, 19-20 and 29-30 min of exercise

⁴ Significantly different from the accumulated exercise trial, P ≤ 0.02
6.3.2 Dietary data

Average energy intakes for the pre-trial day and for day 1 of the trials were 8.10 ± 1.21 MJ (2453 ± 218 kcal) and 7.69 ± 1.24 MJ (2450 ± 225 kcal), respectively. Average dietary intake of fat, carbohydrate and protein was 80.2 ± 10.0 g, 361.4 ± 30.3 g and 103.7 ± 11.7 g respectively, for the pre-trial day, and 76.9 ± 8.8 g, 369.2 ± 31.9 g and 115.8 ± 10.5 g respectively, for day 1.

6.3.3 Plasma concentrations in the fasted state

Fasting plasma concentrations prior to the test meals on day 2 of each trial are shown in Table 6.2. There was no difference in fasting plasma triacylglycerol, glucose and insulin concentrations between trials.
Table 6.2 Fasting plasma concentrations of triacylglycerol (TAG), glucose and insulin for the accumulated exercise, continuous exercise and control trials

<table>
<thead>
<tr>
<th>Trial</th>
<th>TAG (mmol/L)</th>
<th>Glucose (mmol/L)</th>
<th>Insulin (pmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accumulated</td>
<td>0.78 ± 0.07</td>
<td>5.07 ± 0.08</td>
<td>158.5 ± 14.0</td>
</tr>
<tr>
<td>Continuous</td>
<td>0.86 ± 0.05</td>
<td>5.34 ± 0.08</td>
<td>160.0 ± 11.6</td>
</tr>
<tr>
<td>Control</td>
<td>0.87 ± 0.05</td>
<td>5.20 ± 0.97</td>
<td>162.2 ± 14.4</td>
</tr>
</tbody>
</table>

Mean ± SEM; n = 15. Means were compared using one-way ANOVA for the main effect of trial followed by a Bonferroni multiple comparisons test. No significant differences.

6.3.4 Plasma concentrations in the postprandial state

Changes in plasma volume during the observation periods were small and did not differ significantly between trials (accumulated exercise, 0.1 ± 1.8%; continuous exercise, −1.4 ± 2.0%; control, −1.8 ± 1.4%). Thus, plasma concentrations were not adjusted for changes in plasma volume.
Plasma triacylglycerol responses to the test meals are shown in Figure 6.2. Two-way ANOVA revealed significant main effects of trial and time. Plasma triacylglycerol responses were lower on the exercise trials compared with the control trial (post-hoc tests, accumulated exercise versus control, \(P = 0.045\); continuous exercise versus control, \(P = 0.019\)) with little difference between the accumulated and continuous exercise trials. Total and incremental areas under the triacylglycerol concentration versus time curve are given in Table 6.3. There was a main effect of trial for total area under the triacylglycerol concentration versus time curve values. The total area under the plasma triacylglycerol versus time curve was 16% lower on both the accumulated and continuous exercise trials, compared with the control trial. There were no significant differences between the exercise trials for plasma triacylglycerol area under the curve values.
Figure 6.2 Mean (± SEM) fasting and postprandial plasma concentrations of triacylglycerol for accumulated exercise (●), continuous exercise (△) and control (■) trials (n = 15); black rectangles indicate consumption of the test meals. Data were analysed using two-way ANOVA followed by the Bonferroni multiple comparisons test. Main effect of trial (P = 0.005), main effect of time (P < 0.0005).
Table 6.3 Seven-hour areas under the plasma concentration *versus* time curve for total triacylglycerol (TAG) and incremental TAG (adjusted for fasting value) on the accumulated exercise, continuous exercise and control trials \(^1\)

<table>
<thead>
<tr>
<th>Trial</th>
<th>Total TAG (^2) (mmol·7h/L)</th>
<th>Incremental TAG (mmol·7h/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accumulated exercise</td>
<td>9.98 ± 0.67(^3)</td>
<td>4.63 ± 0.46</td>
</tr>
<tr>
<td>Continuous exercise</td>
<td>9.99 ± 0.76(^3)</td>
<td>4.00 ± 0.49</td>
</tr>
<tr>
<td>Control</td>
<td>11.90 ± 1.02</td>
<td>5.64 ± 0.84</td>
</tr>
</tbody>
</table>

\(^1\) Mean ± SEM; \(n = 15\). Means were compared using one-way ANOVA followed by a Bonferroni multiple comparisons test.

\(^2\) Main effect of trial, \(P = 0.003\).

\(^3\) Significantly different from the control trial, \(P < 0.05\).
Plasma glucose and insulin responses to the test meals are shown in Figures 6.3 and 6.4, respectively. No differences were observed between trials for plasma concentrations of glucose and insulin. There was a main effect of time for both glucose and insulin.

Figure 6.3 Mean (± SEM) fasting and postprandial plasma concentrations of glucose for accumulated exercise (●), continuous exercise (△) and control (■) trials (n = 15); the black rectangles indicate consumption of the test meals. Data were analysed using two-way ANOVA followed by the Bonferroni multiple comparisons test. Main effect of time (P < 0.0005).
Figure 6.4 Mean (± SEM) fasting and postprandial plasma concentrations of insulin for accumulated exercise (●), continuous exercise (△) and control (■) trials (n = 15); the black rectangles indicate consumption of the test meals. Data were analysed using two-way ANOVA followed by the Bonferroni multiple comparisons test. Main effect of time (P < 0.0005).

6.4 Discussion

The main finding of this study is that multiple short (3 minute) bouts of brisk walking (30 minutes in total) performed throughout one day are equally effective in reducing postprandial plasma triacylglycerol concentrations as one continuous 30 minute brisk walk. These findings suggest (but do not prove) that accumulating very short bouts of walking may be an effective intervention against cardiovascular disease.
Previous studies have shown that a single bout of prolonged walking (Aldred et al., 1994; Gill et al., 2001a; Gill et al., 2003b; Gill et al., 2001b; Hardman and Aldred, 1995; Katsanos and Moffatt, 2004; Tsetsonis and Hardman, 1996a; Tsetsonis and Hardman, 1996b) and accumulated walking (Murphy et al., 2000) lowers postprandial triacylglycerol concentrations. To author's knowledge, only one study has examined the effects of accumulated brisk walking (three, 10-minute bouts) on postprandial triacylglycerol concentrations (Murphy et al., 2000). However, the present study is the first to examine the effects of accumulating short (<10 minute) bouts of brisk walking on postprandial triacylglycerol concentrations.

For consistency with the current guidelines regarding physical activity and health (Pate et al., 1995; U.K. Department of Health, 2004; U.S. Department of Health and Human Services, 1996), the present study employed moderate levels of activity – both exercise intensity and exercise volume in this study are consistent with the guidelines. This is clearly demonstrated by the low heart rate, percentage of maximal oxygen uptake, RPE, and estimated energy expenditure of 1.10 MJ (265 kcal) elicited by the exercise. However, the participants in this study were young, healthy active men. Thus, the findings may not be applicable to the public at large. Nonetheless, it is reasonable to assume that the volume/intensity of brisk walking performed in the present study is attainable by most individuals regardless of age. Moreover, it may be postulated that a reduction in postprandial lipaemia through accumulated brisk walking may be greater in those who are inactive and/or hypertriacylglycerolaemic since the clearance in triacylglycerol following meals is more likely to be delayed in such individuals (Groot et al., 1991).
One study which supports the notion that the findings of the present study are applicable to the general public is that of Murphy et al. (2000). Their study demonstrated that 30 minutes of brisk walking equivalent to 60% of maximum oxygen uptake accumulated throughout the day (10 minutes × 3) or in one continuous session (30 minutes), performed by sedentary middle-aged individuals reduces postprandial plasma triacylglycerol concentrations measured over a 12-hour period compared with a control trial. The reduction of postprandial triacylglycerol concentrations was comparable in both exercise trials as is the case in the present study. Of note, the percentage reduction in postprandial triacylglycerol concentration in both exercise trials in the study by Murphy et al. (2000) was 12% whereas it was 16% in the present study. It is possible that this difference between studies may be related to the timing of blood collection. Walking was performed on the same day as the test meals were consumed in the study by Murphy et al. (2000) whereas walking was performed the day before the consumption of the test meals in the present study. Thus, the timing in the present study may have maximised skeletal muscle lipoprotein lipase activity and hence enhanced the removal of triacylglycerol (Seip and Semenkovich, 1998). On the basis of the present findings this study provides further support for the concept of accumulation (Pate et al., 1995; U.K. Department of Health, 2004; U.S. Department of Health and Human Services, 1996) and suggests that multiple very short bouts of activity are also likely to be effective for health benefits at least in the case of postprandial lipaemia.

As in the previous studies reported in this thesis the factors contributing to the exercise-induced lowering of postprandial triacylglycerol concentrations are uncertain. Nonetheless, there are two potential mechanisms underlying the reduction of
postprandial triacylglycerol concentrations in the exercise trials relative to the control trial. Increased skeletal muscle lipoprotein lipase activity after aerobic exercise has been proposed as a major factor for the hydrolysis of triacylglycerol from the circulation (Seip and Semenkovich, 1998). However, similar postprandial insulin concentrations throughout the day indicate that muscle lipoprotein lipase activity was possibly similar i.e. the inhibiting effect of insulin on lipoprotein lipase was not modified by exercise. Alternatively, exercise reduces the synthesis and secretion of hepatic VLDL-triacylglycerol (Gill et al., 2001a). Although postprandial insulin concentrations were similar between trials, suggesting that uptake of insulin-mediated fatty acids into adipose tissue was not reduced in the exercise trials, exercise may increase fatty acid oxidation in the liver, leading to a reduced hepatic VLDL-triacylglycerol production.

It has been suggested that only recent exercise appears to facilitate exercise-induced postprandial triacylglycerol lowering effects (Herd et al., 1998; Zhang et al., 2004). Zhang et al (2004) has demonstrated that the reduction of postprandial triacylglycerol concentration was observed when exercise was performed 12 hours prior to the consumption of a test meal but not when the same exercise dose was performed 24 hours prior to the consumption of a test meal. In addition, although cross-sectional studies have shown that trained athletes tend to have a lower postprandial triacylglycerol response relative to sedentary individuals (Tsetsonis et al., 1997), this exercise-induced suppressive effect on postprandial lipaemia is reduced by a short-period of detraining (Gill et al., 2003a; Herd et al., 2000). This emphasises the important role of regular physical activity for the prevention of atherosclerosis. Further research is required to determined whether short bouts of activity, performed on the
same day as test meal(s) are consumed, is effective in lowering postprandial triacylglycerol concentrations. If this is shown to be the case, then additional research could examine whether there is an additive effect on postprandial triacylglycerol concentrations when multiple bouts of exercise are performed on consecutive days.

In conclusion, the results of the present study demonstrate that accumulating 30 minutes of brisk walking in very short bouts is equally effective in reducing postprandial plasma triacylglycerol concentrations on the following day as one continuous 30 minute bout in young men. The present findings regarding the effectiveness of moderate-intensity physical activity in reducing postprandial lipaemia are suggestive of a protective role for accumulated exercise against cardiovascular disease risk. Further research concerning the effects of short bouts of activity, performed on the same day as test meal(s) are consumed, on postprandial triacylglycerol concentrations would be useful from a public health viewpoint.
7.1 Introduction

The previous studies described in this thesis have demonstrated that postprandial triacylglycerol concentration is reduced the day after the accumulation of short bouts of exercise. It is possible that the exercise-induced reductions in postprandial triacylglycerol concentrations in these studies were related to an increase in skeletal muscle lipoprotein lipase activity since its activity is thought to peak >8 hours after exercise (Seip et al., 1997). Therefore, a 2-day model (i.e. exercise on day 1 and postprandial testing on day 2) was chosen in the previous studies in order to maximise the likelihood of a reduction of postprandial triacylglycerol concentration after the exercise trials as opposed to the control trial. However, no studies are available concerning the effect of accumulating short (<10 minute) bouts of activity, performed on the same day as test meals are consumed (1-day model), on postprandial triacylglycerol concentrations.

The first objective of this study was therefore to investigate the effect of accumulating short (5 minute) bouts of running on postprandial plasma triacylglycerol concentrations measured throughout the day on which exercise is performed (not the next day as in previous studies). This study design allowed for an examination of the immediate (same day) effects of exercise on postprandial lipaemia rather than the delayed (next day) effects.
In addition, serum C-reactive protein was measured in this study since it is another emerging cardiovascular disease risk factor. As mentioned earlier C-reactive protein is a marker for inflammation. Recent large prospective studies have reported that elevated plasma C-reactive protein concentrations are associated with increased risk of cardiovascular disease in initially healthy men and women (Ridker et al., 2000; Ridker et al., 1997). Moreover, research has suggested that elevated C-reactive protein may directly contribute to atherosclerosis (Peter et al., 2002). In some studies, exercise training has been shown to lower C-reactive protein concentrations (Kondo et al., 2006; Lakka et al., 2005; Mattusch et al., 2000; Smith et al., 1999; Tisi et al., 1997). In contrast, many studies which have examined the effect of an acute bout of exercise on C-reactive protein concentration report that concentrations are increased 24-48 hours post-exercise (Fallon, 2001; Taylor et al., 1987; Siegel et al., 2001; Weight et al., 1991). However, all of these studies have measured C-reactive protein after prolonged strenuous exercise and such exercise may cause local tissue injury. Nonetheless, one study found no change in C-reactive protein concentration measured 24 hours after a 45 minute walk (Murtagh et al., 2005). In view of this it was postulated that accumulating short bouts of moderate-intensity exercise would not cause an acute phase inflammatory response. Thus, a second objective of this study was to examine the effect of accumulating short (5 minute) bouts of moderate-intensity running on serum C-reactive protein concentrations.

Plasma acylated ghrelin concentrations were also measured in this study. Ghrelin is a novel gastrointestinal hormone and is predominantly secreted by the stomach (Kojima et al., 1999). Ghrelin is involved in short-term appetite regulation, concentrations rise prior to meals (signalling hunger) and decrease after meals (Cummings et al., 2001;
Shiuya et al., 2002). Since exercise influences appetite it is of interest to establish the influence of exercise on ghrelin. As previously mentioned in this thesis, six studies have investigated the effect of an acute bout of aerobic exercise on total plasma ghrelin concentrations (Burns et al., in press; Dall et al., 2002; Kallio et al., 2001; Kraemer et al., 2004a; Schmidt et al., 2001; Zoladz et al., 2005). All of these studies have reported that exercise had no influence on total plasma ghrelin concentrations. These findings suggest that ghrelin may not respond to an exercise induced energy deficit. Alternatively, the lack of change in ghrelin concentration in these studies may relate to the measurement of total ghrelin. Ghrelin exists in two forms - acylated and des-acylated and only acylated ghrelin has biological actions (Hosoda et al., 2000). However, no study has investigated the effect of exercise on plasma acylated ghrelin concentrations. In addition, ghrelin is involved in the short-term regulation of food intake but little is known regarding the influence of exercise on postprandial acylated ghrelin concentrations. This might have important implications regarding the role of exercise in weight control which in turn has implications for cardiovascular disease risk. A third objective of this study was therefore to examine the effect of accumulating short (5 minute) bouts of moderate-intensity running on fasting and postprandial plasma acylated ghrelin concentrations.

Therefore, there were three aims to the present study. The first aim was to investigate the effect of accumulating short (5 minute) bouts of moderate-intensity exercise, performed on the same day as test meals are consumed, on postprandial triacylglycerol concentrations. The second aim was to investigate the effect of accumulating short bouts of moderate-intensity exercise on serum C-reactive protein concentrations and the third aim was to investigate the effect of accumulating short bouts of
moderate-intensity exercise on plasma acylated ghrelin concentrations.

7.2 Methods

7.2.1 Subjects
Ten healthy males (aged 18 to 32 years) volunteered to participate in this study. All subjects were recreationally active and had been weight stable (± 2.5 kg) for at least three months before the study. To minimise risks, subjects were only recruited if they met the following criteria: were non-smoking, were free of known cardiovascular disease or abnormalities, were not taking any medication known to influence lipid or carbohydrate metabolism, had resting arterial blood pressure <140/90 mm/Hg, and had a BMI <35 kg/m². Mean (± SEM) values for the subjects’ age, height, weight, BMI, waist circumference, percentage body fat and maximum oxygen uptake were: 24.4 ± 1.4 y, 176.8 ± 1.8 cm, 71.2 ± 2.1 kg, 22.8 ± 0.6 kg/m², 78.0 ± 1.1 cm, 8.8 ± 0.7 % and 56.0 ± 4.1 mL/kg/min, respectively. The University’s Ethical Advisory Committee approved the study and written informed consent was obtained from all subjects prior to their participation in the study.

7.2.2 Preliminary testing
Anthropometric measurements were made as described in section 3.2. Subjects then performed submaximal (section 3.6.1) and maximal (section 3.6.2) incremental exercise tests to establish the running speed required to elicit 70% of maximum oxygen uptake in the main trials.
7.2.3 Main trials

Each subject underwent two, 1-day trials: an exercise and a control trial. There was a seven-day gap between each trial, and the trials were performed in a randomised design (for the schematic representation of the study protocol, see Figure 7.1).
Exercise trial: subjects performed six (08:30, 10:00, 11:30, 13:00, 14:30 and 16:00), 5 minute runs (at 70% of maximal oxygen uptake) throughout the day with 85 minute rest intervals between bouts.

Control trial: subjects rested in the laboratory throughout the day.

**Note:** Blood samples were collected before performing each bout of exercise.

**Figure 7.1** Schematic representation of the study protocol.
Subjects reported to the laboratory at 08:00 after a 10 hour overnight fast (no food or drink except water). Subjects sat in a semi-supine position on a bed for 10 minutes following arrival at the laboratory. A cannula (Venflon, Becton Dickinson, Helsingborg, Sweden) was then inserted into an antecubital vein and a baseline venous blood sample was collected (section 3.10) at 08:30. Subjects then either performed one, five-minute bout of treadmill running at 70% of maximum oxygen uptake as determined by the preliminary exercise tests or rested before consuming a test meal for breakfast (section 3.9) at 09:00. On the exercise trial, subjects additionally performed five-minute runs at 10:00, 11:30, 13:00, 14:30, and 16:00 (i.e. six, five-minute runs in total). Therefore, there was a 85 minute rest interval after each run. Expired air samples were collected during the last minute of each exercise bout and these were used to determine the actual energy expenditure during exercise (section 3.7). Heart rate was monitored during each bout of exercise using short-range telemetry (section 3.3). Ratings of perceived exertion were assessed periodically during each bout of exercise using the Borg scale (Borg, 1973) (section 3.4). On the control trial, subjects rested throughout the day in the laboratory. During each trial, a second test meal (identical to the first) was consumed at 12:00. Further venous blood samples were collected immediately before each exercise bout or at equivalent time points during the control trial, and one final blood sample was collected at 17:30 in each trial for the measurement of triacylglycerol, glucose and insulin concentrations (section 3.11). For plasma acylated ghrelin measurement (section 3.11.4), blood samples were collected at 08:30, 09:00, 10:00, 10:05, 12:00, 13:00 and 17:30 in each trial. For serum high-sensitivity C-reactive protein measurement (section 3.11.4), blood samples were collected at 08:30 and 17:30 in each trial.
7.2.4 Standardisation of diet and exercise

Subjects weighed and recorded all food and drink consumed for two days prior to the first main trial. Subjects then consumed identical amounts of the same food and drink for two days prior to the second main trial. Thus, meals were standardised between the trials including the evening meal the day before each main trial. Subjects refrained from drinking alcohol during this time. In addition, subjects were asked to remain inactive for two days prior to each main trial and throughout the main trials (other than the exercise performed as part of the experiment). Food diaries were analysed using computerised software (Comp-EAT Version 5.0, Nutrition Systems, London, United Kingdom) to determine energy intake and macronutrient content.

7.2.5 The test meals

Full details of the test meals are given in section 3.9. The average macronutrient content of each test meal was $49.3 \pm 1.5$ g fat, $64.4 \pm 2.0$ g carbohydrate, and $22.2 \pm 0.7$ g protein which provided $3.29 \pm 0.10$ MJ ($786 \pm 24$ kcal) energy (56% fat, 33% carbohydrate and 11% protein). Subjects were asked to consume each meal within 20 minutes. The time taken to consume each meal was recorded and replicated in subsequent trials. Average time taken to consume the first meal (breakfast) was $14.1 \pm 0.9$ minutes. Average time taken to consume the second meal (lunch) was $12.8 \pm 1.1$ minutes. None of the subjects reported nausea or any gastrointestinal discomfort during or after meals. Since the trial order was randomised subjects consumed water *ad libitum* during each main trial. The volume ingested was recorded.

7.2.6 Blood sample analysis

Full details of the blood sample analysis are given in section 3.11. Intra-assay
coefficients of variation were 0.5% for triacylglycerol, 0.8% for glucose, 8.9% for insulin, 4.0% for acylated ghrelin, and 10.7% for high-sensitivity C-reactive protein. Hemoglobin concentration and hematocrit were measured at baseline and the end of the observation period for estimating changes in plasma volume (Dill and Costill, 1974) (section 3.11.1).

7.2.7 Statistical analysis
Data were analysed using the SPSS software version 11.0 for Windows (SPSS Inc, Chicago, U.S.A.). The Shapiro-Wilk test was used to check for normality. Area under the curve values for plasma concentrations over time were calculated using the trapezium rule. Student's *t*-tests for correlated data were used to assess trial differences between fasting plasma/serum concentrations and area under the curve values. Two-way ANOVA with repeated measures was used to examine differences in plasma/serum concentration responses between trials over time. Statistical significance was accepted at the 5% level. Results are presented as mean ± SEM.

7.3 Results
7.3.1 Responses to treadmill running
The total gross energy expenditure during accumulated exercise bouts was 1.76 ± 0.08 MJ (421 ± 18 kcal). Average percentage maximum oxygen uptake and heart rate attained during the accumulated exercise bouts were 72.9 ± 1.7% and 164 ± 3 beats/min, respectively. The median RPE during the exercise bouts was 12 (midway between 'fairly light' and 'fairly hard').
7.3.2 Dietary data

Analysis of food diaries revealed that energy and macronutrient intake did not differ significantly in the 48 hours preceding of the control and exercise trials. Average energy intake two days prior to the trials was $10.1 \pm 0.9$ MJ ($2424 \pm 225$ kcal). Average energy intake one day prior to the trials was $8.4 \pm 0.8$ MJ ($2008 \pm 201$ kcal). Average dietary intake of fat, carbohydrate and protein was $90.8 \pm 18.0$ g, $317.6 \pm 19.8$ g and $110.9 \pm 11.4$ g respectively, two days prior to the trials, and $75.2 \pm 10.5$ g, $255.8 \pm 32.7$ g and $101.1 \pm 26.0$ g respectively, one day prior to the trials.

7.3.3 Plasma and serum concentrations in the fasted state

Plasma and serum concentrations in the fasted state prior to the first test meals on each trial are shown in Table 7.1. There was no difference in fasting plasma triacylglycerol, glucose, insulin, acylated ghrelin and serum high-sensitivity C-reactive protein concentration between the exercise and control trials.
Table 7.1 Fasting concentrations of plasma triacylglycerol (TAG), glucose, insulin, acylated ghrelin and serum high-sensitivity C-reactive protein (hs-CRP) for the exercise and control trials

<table>
<thead>
<tr>
<th>Trial</th>
<th>TAG (mmol/L)</th>
<th>Glucose (mmol/L)</th>
<th>Insulin (pmol/L)</th>
<th>Acylated ghrelin (pg/mL)</th>
<th>hs-CRP (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise</td>
<td>0.99 ± 0.13</td>
<td>5.11 ± 0.14</td>
<td>162.3 ± 29.3</td>
<td>252.0 ± 114.4</td>
<td>0.72 ± 0.27</td>
</tr>
<tr>
<td>Control</td>
<td>0.98 ± 0.12</td>
<td>5.05 ± 0.11</td>
<td>129.4 ± 14.5</td>
<td>322.5 ± 152.0</td>
<td>1.55 ± 1.15</td>
</tr>
</tbody>
</table>

1 Mean ± SEM; n = 10. Means were compared using the Student’s t-tests for correlated data. No significant differences.

7.3.4 Plasma and serum concentrations in the postprandial state

Changes in plasma volume during the observation periods were small (exercise, 0.8±2.5%; control, 0.6±2.7%) and did not differ significantly between trials. Thus, plasma concentrations were not adjusted for changes in plasma volume. Plasma triacylglycerol responses to the test meals are shown in Figure 7.2. Plasma triacylglycerol concentrations were lower on the exercise trial compared with the control trial. The total and incremental areas under the plasma triacylglycerol concentration versus time curve are given in Table 7.2. These were 10% and 29% lower on the exercise trial compared with the control trial, respectively.
Figure 7.2 Mean (± SEM) fasting and postprandial plasma concentrations of triacylglycerol for exercise (○) and control (■) trials (n = 10); black rectangles indicate consumption of the test meals. Data were analysed using two-way ANOVA with repeated measures. Main effect of trial (P = 0.009), main effect of time (P = 0.001).
Table 7.2 Nine-hour areas under the plasma concentration versus time curve for total triacylglycerol (TAG) and incremental TAG (adjusted for fasting value) on the exercise and control trials.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Total TAG (mmol·9h/L)</th>
<th>Incremental TAG (mmol·9h/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise</td>
<td>13.49 ± 1.81&lt;sup&gt;2&lt;/sup&gt;</td>
<td>5.74 ± 1.24&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>15.02 ± 1.92</td>
<td>8.05 ± 1.20</td>
</tr>
</tbody>
</table>

<sup>1</sup> Mean ± SEM; n = 10. Means were compared using the Student’s t-tests for correlated data.

<sup>2</sup> Significantly different from the control trial, P < 0.01.
Plasma glucose, insulin, acylated ghrelin and serum high-sensitivity C-reactive protein responses to the test meals are shown in Figures 7.3, 7.4, 7.5 and 7.6, respectively. Plasma glucose, plasma acylated ghrelin and serum high-sensitivity C-reactive protein concentrations did not differ between trials or over time. Plasma insulin concentrations were lower on the exercise trial compared with the control trial. Plasma insulin concentrations also changed over time.

Figure 7.3 Mean (± SEM) fasting and postprandial plasma concentrations of glucose for exercise (○) and control (■) trials (n = 10); the black rectangles indicate consumption of the test meals. Data were analysed using two-way ANOVA with repeated measures. No significant differences.
Figure 7.4 Mean (± SEM) fasting and postprandial plasma concentrations of insulin for exercise (○) and control (■) trials (n = 10); the black rectangles indicate consumption of the test meals. Data were analysed using two-way ANOVA with repeated measures. Main effect of trial (P = 0.044), main effect of time (P < 0.0005).
Figure 7.5 Mean (± SEM) fasting and postprandial plasma concentrations of acylated ghrelin for exercise (○) and control (■) trials (n = 10); the black rectangles indicate consumption of the test meals. Data were analysed using two-way ANOVA with repeated measures. No significant differences.
Figure 7.6 Mean (± SEM) serum concentrations of high-sensitivity C-reactive protein (hs-CRP) at the beginning (fasting) and end of exercise (○) and control (■) trials (n = 10); the black rectangles indicate consumption of the test meals. Data were analysed using two-way ANOVA with repeated measures. No significant differences.

7.4 Discussion
The primary finding of this study is that postprandial plasma triacylglycerol concentrations are reduced when six, five-minute bouts of running are performed on the same day as test meals are consumed. The secondary finding of this study was that accumulating short bouts of running throughout the day does not alter serum high-sensitivity C-reactive protein concentrations. In addition, the energy deficit created by accumulating short bouts of running throughout the day has no effect on plasma acylated ghrelin concentrations.
Although at least one study has shown that three, 10 minute bouts of brisk walking lower postprandial triacylglycerol concentrations (Murphy et al., 2000), the present study is the first to show that accumulating very short bouts of activity lowers postprandial triacylglycerol concentrations on the same day as the exercise is performed. This is consistent with the data from studies examining postprandial triacylglycerol responses to a single bout of exercise performed on the same day as the postprandial monitoring (Hardman and Aldred, 1995; Katsanos and Moffatt, 2004; Schlierf et al., 1987; Zhang et al., 1998). However, some studies have failed to show an exercise-induced postprandial triacylglycerol lowering effect when exercise is performed on the same day as postprandial monitoring (Petridou et al., 2004; Pfeiffer et al., 2005). The lack of change in postprandial triacylglycerol concentration in these studies may have been due to insufficient energy expenditure during exercise and/or the timing of blood sample collection which may not have continued for long enough after the cessation of exercise to detect differences in triacylglycerol concentration. The mechanism for the exercise-induced lowering of postprandial plasma triacylglycerol concentrations observed in this study may be explained by lowered postprandial insulin concentrations on the exercise trial compared with the control trial. This may contribute to a reduced synthesis and secretion of VLDL-triacylglycerol from the liver as mentioned in previous chapters.

Regarding the magnitude of the reduction in postprandial triacylglycerol concentration, this was smaller in the present study (10% reduction for the total area under the plasma triacylglycerol concentration versus time curve) than in the study reported in Chapter 5 which also involved 30 minutes of accumulated running at 70% of maximal oxygen uptake (22% reduction for the total area under the plasma triacylglycerol concentration.
versus time curve). This suggests that the triacylglycerol lowering effects of exercise begin soon after the cessation of exercise but optimal reductions occur on the next day. An interesting topic for future consideration would be to examine the influence of consecutive days of accumulated activity on postprandial lipaemia measured on consecutive days.

Previous studies concerning C-reactive protein responses following an acute bout of exercise have examined strenuous and prolonged bouts (Fallon, 2001; Siegel et al., 2001; Taylor et al., 1987; Weight et al., 1991). In the present study, the exercise dose may be considered to be of moderate intensity and duration. It was thought that such activity may minimise the acute phase inflammatory response which is often seen with more strenuous activity. The present finding shows that accumulating short bouts of running throughout the day did not alter serum high-sensitivity C-reactive protein concentrations at least measured 9 hours after the first bout of exercise. This finding is consistent with the finding of a previous study which examined a 45 minute bout of walking and measured C-reactive protein concentration 24 hours post-exercise (Murtagh et al., 2005). A limitation of the present study is that further blood samples were not collected after the last bout of exercise. It is possible that serum C-reactive protein concentrations were elevated on the day after exercise in the present study. However, this is speculation and based on the present findings and those of Murtagh et al (2005) mentioned above it seems likely that serum C-reactive protein concentrations are not elevated after exercise of moderate intensity/duration.

In the present study plasma acylated ghrelin concentrations did not differ between trials or over time. This is surprising since it might be expected that the energy deficit
created by accumulating exercise (1.76 MJ = 421 kcal) would result in elevated plasma acylated ghrelin concentrations particularly toward the end of the observation period as has been shown in previous study which involved cycling and measurements of total ghrelin concentration (Christ et al., 2006). However, there was no evidence that plasma acylated ghrelin concentrations were increased at any time-points in the exercise trial in the present study. Furthermore, there were no alterations in plasma acylated ghrelin concentration immediately after a running bout reported in the present study and this is consistent with other studies which have examined the effects of a single bout of aerobic exercise on total ghrelin concentration (Dall et al., 2002; Kallio et al., 2001; Kraemer et al., 2004a; Schmidt et al., 2004; Zoladz et al., 2005). The low exercise volume may explain the lack of alteration in plasma acylated ghrelin concentrations since research has shown that high-intensity running for 1 hour (the total energy expenditure of exercise was 900 kcal) suppresses plasma acylated ghrelin concentration during and immediately post-exercise (David Broom, Loughborough University, PhD student, unpublished observation). Although the precise mechanisms underlying this suppressive effect have not been elucidated, there was an appetite suppression during and immediately following high-intensity running as determined by measurements using a ‘hunger scale’. Further research is required to investigate the role of acylated ghrelin in exercise metabolism.

Previous studies have shown that total plasma ghrelin concentrations are reduced after a meal (Callahan et al., 2004; Cummings et al., 2001). In the present study, there is no clear fall in plasma acylated ghrelin concentrations in either the exercise or the control trial following meal consumption. The lack of change in plasma acylated ghrelin concentrations following a fat-rich meal is consistent with the findings of a previous
study which measured plasma acylated ghrelin (Tentolouris et al., 2004). However, the lack of change in plasma acylated ghrelin concentration in the present study may have been due to the fact that acylated ghrelin was measured one hour after eating in the present study whereas in a previous study which did demonstrate acylated ghrelin suppression after feedings plasma acylated ghrelin was measured within 30 minutes of eating (Al Awar et al., 2005). In addition, one study has shown that meal-induced suppression of plasma acylated ghrelin only observed after a carbohydrate-rich meal and not in an isoenergetic fat-rich meal (Tentolouris et al., 2004), suggesting that the high-fat meals employed in the present study may not lead to changes in plasma acylated ghrelin concentration.

In the present study, energy intake (kcal/day) on the exercise trial was matched to that of the control trial – hence subjects were in energy deficit on the exercise trial. It is possible that exercise-induced reductions in postprandial plasma triacylglycerol concentrations were related to a negative energy balance since in this state adipose lipoprotein lipase activity is increased (Taskinen and Nikkila, 1987). Further research examining the influence of accumulated short bouts of exercise on postprandial triacylglycerol concentrations while ensuring that energy intake is increased to compensate for the energy expended during exercise would be useful. This would indicate whether the accumulated exercise-induced lowering of postprandial plasma triacylglycerol concentration is mediated by the energy deficit created by exercise or the exercise per se.

In conclusion, accumulating short (5 minute) bouts of running throughout the day for a total of 30 minutes is effective in reducing postprandial plasma triacylglycerol
concentrations measured on the same day in young men. In contrast, there was no alteration in serum high-sensitivity C-reactive protein concentrations on the accumulated exercise trial indicating that such exercise does not lead to acute-phase inflammatory response. In addition, plasma acylated ghrelin concentrations did not differ between trials, suggesting that plasma acylated ghrelin concentration may not be responsive to the accumulation of short bouts of exercise. Further research is required to investigate whether accumulated exercise reduces postprandial triacylglycerol concentrations even when food intake is increased to compensate for the energy expended during exercise (i.e. in the state of energy balance). Further research is also required to determine if C-reactive protein concentrations are altered on the day after accumulated exercise bouts and further research is warranted regarding the response of acylated ghrelin to accumulated activity. Such research should involve more frequent measurement of acylated ghrelin since changes were possibly missed in the present study due to the relatively infrequent sampling protocol.
CHAPTER 8
THE EFFECT OF ACCUMULATED EXERCISE ON RESTING BLOOD PRESSURE

8.1 Introduction

Although high blood pressure (a systolic blood pressure of 140 mm Hg or over, and/or a diastolic blood pressure of 90 mm Hg or over) is associated with an increased risk of cardiovascular disease, a recent report has introduced a new classification, the term 'prehypertension' (a systolic blood pressure ranging from 120-139 mm Hg and a diastolic blood pressure ranging from 80-89 mm Hg) that requires early adoption of lifestyle modifications to prevent further increases in blood pressure and cardiovascular disease (Chobanian et al., 2003). In the United Kingdom, one in three adults have hypertension or are being treated for hypertension (Petersen et al., 2004). Similarly nearly one in three adults has high blood pressure in the United States (Fields et al., 2004). Regular physical activity as a non-pharmacological approach is recommended for the prevention, control and treatment of hypertension with minimum cost and side effects (Pescatello et al., 2004).

Recent public health guidelines on physical activity emphasise that adults should accumulate 30 minutes of moderate intensity activity on most, preferably all, days of the week. Moreover, a minimum of 10-minutes is recommended for any one bout of activity (Pate et al., 1995). Chronic training studies have shown that several short bouts of accumulated activity are at least as effective in reducing blood pressure as one, continuous bout of activity (Coleman et al., 1999; Staffileno et al., 2001). However, it has been suggested that training-induced blood pressure lowering may simply reflect
acute postexercise hypotension (Seals et al., 1997), highlighting the importance of regular exercise. Postexercise hypotension, a reduction in resting blood pressure lasting several hours after exercise, has been widely reported in both normotensive (Lockwood et al., 2005; MacDonald et al., 2000) and hypertensive (Brandao Rondon et al., 2002; Quinn, 2000) individuals, but the largest reduction is often seen in hypertensive individuals (Hagberg et al., 1987).

Although significant research attention has been given to the acute effect of a single bout of aerobic exercise on blood pressure, only three studies have investigated the acute effect of short (10-15 minute) bouts of intermittent exercise (Hagberg et al., 1987; Kaufman et al., 1987) and lifestyle physical activity throughout the day (Padilla et al., 2005) on blood pressure. Moreover, the acute effect of a total of 30 minutes of accumulated exercise in bouts shorter than 10 minutes per session throughout the day compared with one equal volume of continuous exercise on blood pressure has not been determined.

The findings presented in this chapter are important for two reasons. Firstly, a previous study indicates that even amongst people who follow the recommended physical activity guidelines of 30 minutes on most if not all days of the week (Pate et al., 1995) most people do not exercise continuously for 10 minutes at a time (Whitt et al., 2003), therefore the partitioning of exercise sessions into multiple short bouts throughout the day may be easier to fit into everyday life. Secondly, it has been suggested that small increases in blood pressure in healthy, normotensive men and women are linked to the risk of developing hypertension (Blair et al., 1984). Investigation of the effects of exercise on blood pressure in normotensive populations is therefore as clinically
important as it is in hypertensive individuals.

The blood pressure data presented in this chapter were collected during the studies in Chapters 4, 5 and 6. Part I examines the blood pressure response to the accumulation of a 1000 kcal energy expenditure in 6 minute running bouts completed throughout the day. Part II compares blood pressure responses to running exercise performed either in one continuous 30 minute bout or accumulated in ten, 3-minute bouts performed throughout one day. Finally, Part III is a repetition of the protocol employed in Part II except the mode of exercise walking rather than running. In Parts II and III the energy expenditure of the continuous and accumulated bouts was matched i.e. the total energy expenditure was the same on continuous and accumulated exercise trials.

8.2 Methods

Part I - Blood pressure responses to the accumulation of 1000 kcal in 6 minute bouts of running

8.2.1 Main trials

Full details of the method are given in Chapter 4 (section 4.3). Briefly, each subject underwent three, 2-day trials: an accumulated exercise trial, a continuous exercise trial, and a control trial separated by at least an interval of seven-days in a randomised, repeated measures design. Subjects were asked to remain inactive on the day before day 1 of each trial and throughout the main trials (other than the exercise performed as part of the experiment). (for the schematic representation of the study protocol, see Figure 8.1).
Exercise trial: subjects performed 6-minute runs (at 70% of maximal oxygen uptake) throughout the day with 30 minute rest intervals between bouts. This continued until subjects had accumulated a gross energy expenditure of 4.2 MJ (1000 kcal). This required between 9 and 15 bouts depending on the subject.

Control trial: subjects rested in the laboratory throughout the day.

Consumption of packed lunch

Note.
Day 1: resting blood pressure was measured 15 minutes after the termination of each exercise bout or at an equivalent time point during the control trial.

Figure 8.1 Schematic representation of the study protocol
Day 1. On day 1 of each trial, subjects reported to the laboratory between 08:30 and 09:00 having eaten breakfast. Upon arrival in the laboratory subjects were asked to sit quietly for 10 minutes after which resting arterial blood pressure was measured (section 3.5). On the control trial subjects were required to continue resting throughout the day in the laboratory. On the exercise trial subjects performed 6 minute bouts of treadmill running throughout the day with a 30 minute rest interval between each bout. The exercise intensity for the running bouts was set at 70% of maximum oxygen uptake as determined by the preliminary exercise tests (sections 3.6.1 and 3.6.2). This pattern continued until subjects had accumulated a gross energy expenditure of 4.2 MJ (1000 kcal). This required between 9 and 15 bouts depending on the subject. Resting arterial blood pressure was measured 15 minutes after the termination of each exercise bout (or at an equivalent time point during the control trial). Subjects consumed a packed lunch midway through the day. Subjects left the laboratory between 16:00 and 17:00 and they were instructed to consume an early evening meal and rest for the remainder of the evening.

Day 2. On day 2 of each trial subjects reported to the laboratory between 08:00 and 08:30 after a 10 hour overnight fast (no food or drink except water). Following cannulation baseline resting blood pressure was measured. Then a standardised breakfast was served (section 3.9). After this, resting blood pressure was measured at hourly intervals for seven hours. A standardised lunch was also provided three hours after consuming breakfast.

8.2.2 Arterial blood pressure

Resting arterial blood pressure was measured by auscultation using a mercury
sphygmomanometer (Accoson freestyle stand 0042, CardioHinetics, Salford, United Kingdom). Subjects were seated in a chair for 5 minutes before measurements. Two measurements were taken at each time point and the mean of these values recorded. Since blood pressure was measured 15 minutes after each exercise bout the total number of post-exercise recordings for day 1 varied from 9 to 15 depending on the subject. For day 2 the total number of blood pressure recordings was eight.

8.2.3 Statistical analysis

Data were analysed using the SPSS software version 11.0 for Windows (SPSS Inc, Chicago, U.S.A.). The Shapiro-Wilk test was used to check for normality. Area under the curve values for blood pressure versus time were calculated using the trapezium rule. Student’s t-tests for correlated data were used to assess trial differences between area under the curve values. Statistical significance was accepted at the 5% level. Results are presented as mean ± SEM.

Part II - Blood pressure responses to the accumulation of 30 minutes of running in 3 minute bouts

8.2.4 Main trials

Full details of the method are given in Chapter 5 (section 5.3). Briefly, each subject underwent three, 2-day trials: an accumulated exercise trial, a continuous exercise trial, and a control trial separated by at least an interval of seven-days in a randomised, repeated measures design. Subjects were asked to remain inactive on the day before day 1 of each trial and throughout the main trials (other than the exercise performed as
part of the experiment). (for the schematic representation of the study protocol, see Figure 8.2).
Accumulated exercise trial: subjects performed ten, 3-minute runs (at 70% of maximal oxygen uptake) throughout the day with 30-minute rest intervals between bouts.

Continuous exercise trial: subjects performed one, 30-minute run (at 70% of maximal oxygen uptake) in the afternoon (15:00-15:30).

Control trial: subjects rested in the laboratory throughout the day.

**Note.**
Day 1: resting blood pressure was measured immediately and 15 minutes after the termination of each bout of exercise in the accumulated exercise trial or at an equivalent time point during the continuous exercise and control trial.

**Figure 8.2** Schematic representation of the study protocol
Day 1. On day 1 of each trial, subjects reported to the laboratory at 09:00 having eaten breakfast. Upon arrival in the laboratory subjects were asked to sit quietly in a chair for 20 minutes and then resting arterial blood pressure (section 3.5) and heart rate (section 3.3) were measured. A clock was then started at 09:30 and stopped at 12:00 for the morning session and started again at 13:15 and stopped at 15:45 for the afternoon session. On the accumulated exercise trial subjects performed ten, three-minute bouts of running on the treadmill throughout the day separated by intervals of 30 minutes, except for the lunch break which lasted 75 minutes (12:00-13:15). Blood pressure and heart rate were measured in a seated position immediately after each bout of exercise. Blood pressure and heart rate were also measured in a seated position 15 minutes after the termination of each exercise bout. These blood pressure and heart rate measurements were made at equivalent time points during the continuous exercise and control trials. On the continuous exercise trial subjects performed one 30 minute run on the treadmill in the afternoon from 15:00-15:30. Of note in the continuous exercise trial, resting blood pressure and heart rate measured at the equivalent of the 9th period in the accumulated exercise trial were measured slightly early to allow time for the 30 minute run. For both exercise trials, exercise was designed to end at 15:30 so that the time interval between the cessation of exercise and baseline blood pressure measurements on day 2 was the same (17 hours). The exercise intensity for treadmill running was set at 70% of maximum oxygen uptake as determined by the preliminary exercise tests (section 3.6.1 and 3.6.2). On the control trial subjects were required to continue resting throughout the day in the laboratory. During each trial, subjects consumed a packed lunch midway through the day. Subjects left the laboratory at approximately 16:00 and they were instructed to rest for the remainder of the evening at home.
Day 2. On day 2 of each trial subjects reported to the laboratory at 08:00 after a 10 hour overnight fast (no food or drink except water). Following cannulation baseline resting blood pressure and heart rate were measured. Then a standardised breakfast was served (section 3.9). After this, blood pressure was measured at hourly intervals for seven hours. A standardised lunch was also provided three hours after consuming breakfast.

8.2.5 Arterial blood pressure

Arterial blood pressure was measured in a seated position by auscultation using a random-zero sphygmomanometer (Hawksley Mk. II, Hawksley and Sons Ltd., Sussex, United Kingdom). Subjects were seated on a chair for 5 minutes before measurements (except for measurements immediately after exercise). Two measurements were taken at each time point and the mean of these values recorded.

8.2.6 Statistical analysis

Data were analysed using the SPSS software version 12.0 for Windows (SPSS Inc, Chicago, U.S.A.). The Shapiro-Wilk test was used to check for normality. One-way ANOVA was used to assess the main effect of trial (with Bonferroni post-hoc multiple comparisons tests) for baseline blood pressure data on day 1 and 2. Two-way ANOVA with repeated measures followed by the Bonferroni post-hoc multiple comparisons test was used to examine trial differences over time for blood pressure. Statistical significance was accepted at the 5% level. Results are presented as mean ± SEM.
Part III - Blood pressure responses to the accumulation of 30 minutes of walking in 3 minute bouts

8.2.7 Main trials
Full details of the methods are given in Chapter 6 (section 6.3). The experimental design of Part III was identical to the experimental design of Part II except that a) the mode of exercise was walking (at a self-selected brisk pace) and b) additional blood pressure measurements were collected (in a seated position) 5 minutes post-exercise (or at an equivalent time point in the continuous exercise and control trials). Blood pressure measurements were performed in the same was as reported in Part II and the statistical analysis employed was identical to that employed in Part III.

8.3 Results
Part I
8.3.1 Blood pressure responses to the accumulation of 1000 kcal in 6 minute bouts of running
Area under the curve values for blood pressure versus time on days 1 and 2 are shown in Table 8.1. Total area under the curve values have been divided by the number of observations so that values are meaningful. On day 1, systolic and diastolic blood pressures were lower for the exercise trial compared with the control trial. On day 2 systolic blood pressure, but not diastolic blood pressure, was lower for the exercise trial compared with the control trial.
Table 8.1 Mean systolic and diastolic blood pressure values (total area under the pressure versus time curve divided by the number of observations) for the exercise and control trials

<table>
<thead>
<tr>
<th>Trial</th>
<th>Systolic blood pressure</th>
<th>Diastolic blood pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DAY 1</td>
<td>DAY 2</td>
</tr>
<tr>
<td></td>
<td>mm Hg</td>
<td>mm Hg</td>
</tr>
<tr>
<td>Exercise</td>
<td>115.2 ± 1.9&lt;sup&gt;2&lt;/sup&gt;</td>
<td>117.4 ± 1.9&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>120.6 ± 1.6</td>
<td>120.4 ± 1.6</td>
</tr>
</tbody>
</table>

<sup>1</sup> Mean ± SEM; n = 19. Means were compared using the Student’s t-test for correlated data.

<sup>2</sup> Significantly different from the control trial, P < 0.0005.

<sup>3</sup> Significantly different from the control trial, P = 0.003.

<sup>4</sup> Significantly different from the control trial, P = 0.035.

Part II

8.3.2 Blood pressure responses to the accumulation of 30 minutes of running in 3 minute bouts

Table 8.2 shows mean systolic and diastolic blood pressure measured on day 1 and day 2 for each trial. Baseline resting systolic blood pressure and diastolic blood pressure measured on day 1 did not differ between the trials (Table 8.2). There was a significant difference in systolic blood pressure measured immediately after each bout of running during the accumulated running trial or at equivalent time points during the continuous
running and control trials (Table 8.2) (main effect of trial, $P < 0.0005$; main effect of time, $P < 0.0005$; trial × time interaction, $P < 0.0005$). Post-hoc multiple comparisons for the main effect of trial revealed that systolic blood pressure was significantly higher on the accumulated running trial compared with the continuous running and control trials (accumulated running versus continuous running, $P < 0.0005$; accumulated running versus control, $P < 0.0005$) (Table 8.2). There was no difference between the continuous running and control trials for systolic blood pressure. There was no difference in diastolic blood pressure measured immediately after each bout of exercise during the accumulated running trial or at equivalent time points during the continuous running and control trials (Table 8.2).

There was a significant difference in systolic blood pressure measured at 15 minutes after the termination of each bout of running during the accumulated running trial or at equivalent time points during the continuous running and control trials (Table 8.2) (main effect of trial, $P < 0.0005$; main effect of time, $P = 0.007$; trial × time interaction, $P = 0.064$). Post-hoc multiple comparisons for the main effect of trial revealed that systolic blood pressure was significantly lower during the accumulated running trial compared with the continuous running and control trials (accumulated running versus continuous running, $P = 0.002$; accumulated running versus control, $P = 0.002$) (Table 8.2). There was no difference between continuous running and control trials for systolic blood pressure. There was no difference in diastolic blood pressure measured 15 minutes after the termination of each bout of exercise during the accumulated running trial or at equivalent time points during the continuous running and control trials (Table 8.2). A graphical representation of the day 1 blood pressure responses to ten, 3-minute runs is illustrated in Figures 8.3 and 8.4.
Table 8.2 Mean systolic blood pressure (SBP) and diastolic blood pressure (DBP) measured on day 1 and day 2 of the accumulated running, continuous running and control trials

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Day 1</td>
</tr>
<tr>
<td></td>
<td>Immediate post-running$^a,^1$</td>
<td>15 minutes post-running$^a,^1$</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accumulated running</td>
<td>118 ± 2</td>
<td>155 ± 4$^2$</td>
</tr>
<tr>
<td>Continuous running</td>
<td>117 ± 2</td>
<td>120 ± 2</td>
</tr>
<tr>
<td>Control</td>
<td>119 ± 2</td>
<td>117 ± 2</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accumulated running</td>
<td>66 ± 2</td>
<td>72 ± 2</td>
</tr>
<tr>
<td>Continuous running</td>
<td>67 ± 2</td>
<td>70 ± 2</td>
</tr>
<tr>
<td>Control</td>
<td>68 ± 2</td>
<td>71 ± 1</td>
</tr>
</tbody>
</table>

$^a$ Means of 10 post-exercise measurements i.e. one for each bout of exercise. $^b$ Means of 8 measurements taken at one hour intervals throughout day 2.

$^1$ Main effect of trial, $P \leq 0.001$. $^2$ Significantly different from the continuous running and control trials, $P \leq 0.002$. $^3$ Significantly different from the control trial, $P \leq 0.007$. 

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Figure 8.3 Day 1 mean (± SEM) systolic blood pressure (SBP) and diastolic blood pressure (DBP) responses immediately after each bout of running during the accumulated running trial (●), or at equivalent time points during the continuous running (△) and control (■) trials (n = 10); a black rectangle indicates consumption of lunch. Note. No exercise was performed between observation 1 and observation 9 in the continuous running trial. Data were analysed using two-way ANOVA followed by the Bonferroni multiple comparisons test. SBP: main effect of trial (P < 0.0005), main effect of time (P < 0.0005), main effect of trial × time interaction (P < 0.0005). DBP: no significant differences.
Figure 8.4 Day 1 mean (± SEM) systolic blood pressure (SBP) and diastolic blood pressure (DBP) responses 15 minutes after the termination of each bout of running during the accumulated running trial (●), or at equivalent time points during the continuous running (▲) and control (■) trials (n = 10); a black rectangle indicates consumption of lunch. Note. No exercise was performed between observation 1 and observation 9 in the continuous running trial. Data were analysed using two-way ANOVA followed by the Bonferroni multiple comparisons test. SBP: main effect of trial (P < 0.0005), main effect of time (P = 0.007). DBP: no significant differences.

Figure 8.5 shows resting systolic and diastolic blood pressure measured throughout day 2. There was a significant difference in baseline resting systolic blood pressure measured on day 2 between the trials (main effect of trial, P = 0.001). Post-hoc multiple comparisons for the main effect of trial revealed that baseline resting systolic blood pressure measured on day 2 was significantly lower on the accumulated (108 ± 2
mm Hg, $P < 0.0005$) and continuous running trials ($109 \pm 2$ mm Hg, $P = 0.018$) compared with the control trial ($116 \pm 2$ mm Hg). There was no difference between the accumulated running and continuous running trials for baseline resting systolic blood pressure on day 2. There were no significant differences for baseline resting diastolic blood pressure between trials on day 2. There was a significant difference between trials on resting systolic blood pressure measured throughout day 2 (Table 8.2 and Figure 8.5) (main effect of trial, $P = 0.001$; main effect of time, $P = 0.006$; trial $\times$ time interaction, $P = 0.371$). Post-hoc multiple comparisons for the main effect of trial revealed that resting systolic blood pressure measured throughout day 2 was significantly lower on the accumulated and continuous running trials compared with the control trial (accumulated running versus control exercise, $P < 0.0005$; continuous running versus control, $P = 0.007$) (Table 8.2 and Figure 8.5). There was no difference between the accumulated and continuous running trials in resting systolic blood pressure measured throughout day 2. There was no difference in resting diastolic blood pressure measured throughout day 2 between the three trials (Table 8.2 and Figure 8.5).
Figure 8.5 Mean (± SEM) resting systolic blood pressure (SBP) and diastolic blood pressure (DBP) measured on day 2 of the accumulated running (●), continuous running (△) and control ( ■) trials (n = 10); black rectangles indicate consumption of the meals. Data were analysed using two-way ANOVA followed by the Bonferroni multiple comparisons test. SBP: main effect of trial (P = 0.001), main effect of time (P = 0.006). DBP: main effect of time (P < 0.0005).

Part III

8.3.3 Blood pressure responses to the accumulation of 30 minutes of walking in 3 minute bouts

Table 8.3 shows mean systolic and diastolic blood pressure measured on day 1 and day 2 for each trial. Baseline resting systolic and diastolic blood pressure measured on day 1 were not different between the trials (Table 8.3). There was a significant difference in systolic blood pressure measured immediately after each bout of walking during the
accumulated walking trial or at equivalent time points during the continuous walking and control trials (Table 8.3) (main effect of trial, $P < 0.0005$; main effect of time, $P < 0.0005$; trial × time interaction, $P < 0.0005$). Post-hoc multiple comparisons for the main effect of trial revealed that systolic blood pressure was significantly higher on the accumulated walking trial compared with the continuous walking and control trials (accumulated walking versus continuous walking, $P < 0.0005$; accumulated exercise versus control, $P < 0.0005$) (Table 8.3). There was no difference between the continuous walking and control trials for systolic blood pressure. There was no difference in diastolic blood pressure measured immediately after each bout of walking during the accumulated exercise trial or at equivalent time points during the continuous walking and control trials (Table 8.3).

There was no difference in systolic and diastolic blood pressure measured 5 minutes after the termination of each bout of exercise during the accumulated walking trial or at equivalent time points during the continuous walking and control trials (Table 8.3).

There was a significant difference in systolic blood pressure measured 15 minutes after the termination of each bout of walking during the accumulated walking trial or at equivalent time points during the continuous walking and control trials (Table 8.3) (main effect of trial, $P < 0.0005$; main effect of time, $P = 0.060$; trial × time interaction, $P = 0.130$). Post-hoc multiple comparisons for the main effect of trial revealed that systolic blood pressure were significantly lower in the accumulated walking trial compared with the continuous walking and control trials (accumulated walking versus continuous walking, $P < 0.0005$; accumulated walking versus control, $P < 0.0005$) (Table 8.3). There was no difference between the continuous walking and control trials.
for systolic blood pressure. There was no difference in diastolic blood pressure measured 15 minutes after the termination of each bout of walking during the accumulated walking trial or at equivalent time points during the continuous walking and control trials (Table 8.3). A graphical representation of the day 1 blood pressure responses to ten, 3-minute walks is illustrated in Figures 8.6, 8.7 and 8.8.
Table 8.3 Mean systolic blood pressure (SBP) and diastolic blood pressure (DBP) measured on day 1 and day 2 of the accumulated walking, continuous walking and control trials

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th></th>
<th>Day 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post-walking&lt;sup&gt;a,1&lt;/sup&gt;</td>
<td>5 minutes</td>
<td>15 minutes</td>
</tr>
<tr>
<td><strong>SBP (mm Hg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accumulated walking</td>
<td>117 ± 1</td>
<td>140 ± 1&lt;sup&gt;2&lt;/sup&gt;</td>
<td>114 ± 1</td>
<td>109 ± 1&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Continuous walking</td>
<td>114 ± 2</td>
<td>117 ± 1</td>
<td>115 ± 2</td>
<td>113 ± 2</td>
</tr>
<tr>
<td>Control</td>
<td>115 ± 2</td>
<td>115 ± 2</td>
<td>114 ± 1</td>
<td>114 ± 1</td>
</tr>
<tr>
<td><strong>DBP (mm Hg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accumulated walking</td>
<td>67 ± 2</td>
<td>67 ± 2</td>
<td>69 ± 2</td>
<td>67 ± 2</td>
</tr>
<tr>
<td>Continuous walking</td>
<td>68 ± 2</td>
<td>68 ± 2</td>
<td>68 ± 2</td>
<td>68 ± 2</td>
</tr>
<tr>
<td>Control</td>
<td>67 ± 2</td>
<td>68 ± 2</td>
<td>69 ± 2</td>
<td>69 ± 2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Means of 10 post-exercise measurements i.e. one for each bout of exercise. <sup>b</sup> Means of 8 measurements taken at one hour intervals throughout day 2.

<sup>1</sup> Main effect of trial, \( P < 0.0005 \).

<sup>2</sup> Significantly different from the continuous exercise and control trials, \( P < 0.0005 \).

<sup>3</sup> Significantly different from the control trial, \( P < 0.0005 \).
Figure 8.6 Day 1 mean (± SEM) systolic blood pressure (SBP) and diastolic blood pressure (DBP) responses immediately after each bout of walking during the accumulated walking trial (●), or at equivalent time points during the continuous walking (△) and control (■) trials (n = 15); a black rectangle indicates consumption of lunch. Note. No exercise was performed between observation 1 and observation 9 in the continuous walking trial. Data were analysed using two-way ANOVA followed by the Bonferroni multiple comparisons test. SBP: main effect of trial (P < 0.0005), main effect of time (P < 0.0005), main effect of trial × time interaction (P < 0.0005). DBP: no significant differences.
Figure 8.7 Day 1 mean (± SEM) systolic blood pressure (SBP) and diastolic blood pressure (DBP) responses 5 minutes after the termination of each bout of walking during the accumulated walking trial (●), or at equivalent time points during the continuous walking (△) and control (■) trials (n = 15); a black rectangle indicates consumption of lunch. Note. No exercise was performed between observation 1 and observation 9 in the continuous walking trial. Data were analysed using two-way ANOVA followed by the Bonferroni multiple comparisons test. SBP: no significant differences. DBP: no significant differences.
Figure 8.8 Day 1 mean (± SEM) systolic blood pressure (SBP) and diastolic blood pressure (DBP) responses 15 minutes after the termination of each bout of walking during the accumulated walking trial (●), or at equivalent time points during the continuous walking (△) and control (■) trials (n = 15); a black rectangle indicates consumption of lunch. Note. No exercise was performed between observation 1 and observation 9 in the continuous walking trial. Data were analysed using two-way ANOVA followed by the Bonferroni multiple comparisons test. SBP: main effect of trial (P < 0.0005), main effect of time (P = 0.006). DBP: no significant differences.

Figure 8.9 shows resting systolic and diastolic blood pressure measured throughout day 2. There was a significant difference in baseline resting systolic blood pressure measured on day 2 between the trials (main effect of trial, P < 0.0005). Post-hoc multiple comparisons for the main effect of trial revealed that baseline resting systolic
blood pressure measured on day 2 was significantly lower on the accumulated (107 ± 1 mm Hg, \( P < 0.0005 \)) and continuous walking trials (107 ± 1 mm Hg, \( P < 0.0005 \)) compared with the control trial (115 ± 1 mm Hg). There was no difference between the accumulated walking and continuous walking trials for baseline resting systolic blood pressure on day 2. There was a significant difference in baseline resting diastolic blood pressure measured on day 2 between the trials (main effect of trial, \( P = 0.017 \)). Post-hoc multiple comparisons for the main effect of trial revealed that baseline resting diastolic blood pressure measured on day 2 was significantly lower on the accumulated walking trial (109 ± 1 mm Hg, \( P < 0.0005 \)) compared with the control trial (117 ± 1 mm Hg). Baseline resting diastolic blood pressure tended to be lower on the continuous walking trial (110 ± 1 mm Hg) compared with the control trial but the difference was not significant (\( P = 0.074 \)). There was a significant difference between trials in resting systolic blood pressure measured throughout day 2 (Table 8.3 and Figure 8.9) (main effect of trial, \( P < 0.0005 \); main effect of time, \( P = 0.003 \); trial \( \times \) time interaction, \( P = 0.561 \)). Post-hoc multiple comparisons for the main effect of trial revealed that resting systolic blood pressure measured throughout day 2 was significantly lower on the accumulated and continuous walking trials compared with the control trial (accumulated walking \textit{versus} control exercise, \( P < 0.0005 \); continuous walking \textit{versus} control, \( P < 0.0005 \)) (Table 8.3 and Figure 8.9). There was no difference between the accumulated and continuous walking trials in resting systolic blood pressure measured throughout day 2. There was no difference in resting diastolic blood pressure measured throughout day 2 between the three trials (Table 8.3 and Figure 8.9).
8.4 Discussion

The present study demonstrates that resting systolic and diastolic blood pressure were lower in periods between 6 minute bouts of exercise in comparison to equivalent time points in the absence of exercise. Moreover, accumulated exercise induced lowering of systolic blood pressure was maintained for up to 24 hours after exercise (Part I). Furthermore, the present study shows that accumulating 30 minutes of walking or
running throughout the day or in a single 30 minute session equally reduce systolic blood pressure measured on the following day (Parts II and III). The novel finding in this study is that accumulating very short (3 minute) bouts of exercise reduces systolic blood pressure measured throughout the following day in healthy young normotensive men.

Resting systolic and diastolic blood pressure were reduced in the periods between exercise bouts throughout day 1 when 6 minute bouts of exercise were performed (Part I). The average reductions were 5 mm Hg for systolic blood pressure and 3 mm Hg for diastolic blood pressure in comparison with the control trial. Blood pressure was not measured during exercise in the first study (Part I), but it should be recognised that systolic blood pressure probably increased during this time. Another major finding in studies 2 and 3 was that systolic blood pressure measured 15 minutes after each 3 minute bout of walking/running in the accumulated exercise trials was reduced significantly compared with equivalent time points during the control trial. This finding agrees with that of a previous study of postexercise hypotension in which systolic blood pressure, but not diastolic blood pressure, was lower 60 minutes postexercise compared with values on a non-exercise control trial in young, normotensive subjects after intermittent bouts of walking (5 × 10-minutes) (Kaufman et al., 1987). Similarly, another study using older hypertensive subjects performing three, 15-minute treadmill runs at 70% of maximal oxygen uptake showed that systolic blood pressure, but not diastolic blood pressure, was lower for up to 2 hours after exercise (Hagberg et al., 1987). In addition, the present finding of a significant reduction in systolic blood pressure measured following 15 minutes after exercise in the continuous exercise trial is consistent with earlier reports of postexercise
hypotension in normotensive individuals after a single bout of moderate intensity exercise (Forjaz et al., 2004) and maximum intensity exercise (Piepoli et al., 1993).

Although the mechanism responsible for acute exercise-induced blood pressure lowering may be a persistent reduction of vascular resistance through the reduction in sympathetic activity and enhanced peripheral vasodilation, the later hypothesis of increased vasodilator substances has been suggested to play a major role for postexercise hypotension (Halliwill, 2001). Indeed, a heart rate above the baseline level observed at 15 minutes after the termination of exercise in the accumulated and continuous exercise trials (Part II: accumulated running trial, baseline 65 ± 3, 15-minutes post-running, 77 ± 3; continuous running trial, baseline, 65 ± 2, 15-minutes post-running, 98 ± 2. Part III: accumulated walking trial, baseline 70 ± 2, 15-minutes post-walking, 72 ± 2; continuous walking trial, baseline, 67 ± 2, 15-minutes post-walking, 80 ± 3) in the present study indicates that postexercise sympathoinhibition may not be the main factor responsible for postexercise hypotension. It is also possible that reduced insulin concentrations contribute to the blood pressure lowering effect of exercise since hyperinsulinemia is associated with hypertension (Reaven, 1994).

Baseline systolic blood pressure (but not diastolic blood pressure) was also reduced (by 3 mm Hg on average) the day after accumulated activity in the present study compared with baseline systolic blood pressure on day 1 i.e. before any exercise was performed (Part I). In addition, in comparison with baseline systolic blood pressure measured on day 1, baseline systolic blood pressure (but not diastolic blood pressure) was reduced (by 10 mm Hg and 8 mm Hg in the accumulated and continuous running trials,
respectively) on day 2 in both exercise trials in the study reported in Part II and reduced (by 11 mm Hg and 7 mm Hg in the accumulated and continuous walking trials, respectively) on day 2 in both exercise trials in the study reported in Part III. The studies reported here also show that exercise-induced lowering of systolic blood pressure was sustained for at least 24 hours after the last bout of exercise (Parts I, II and III). The present data extend the findings of previous reports that postexercise hypotension is sustained for up to 6 hours in middle-aged prehypertensive and up to 8 hours in older hypertensive individuals following accumulated activity (Padilla et al., 2005), and for 24 hours following a single bout of exercise in older hypertensive individuals (Quinn, 2000; Taylor-Tolbert et al., 2001). However, some studies have found that a single session of exercise has no prolonged effect on postexercise blood pressure in both normotensive (Brandao Rondon et al., 2002) and hypertensive individuals (Somers et al., 1991) or has detrimental effects on subsequent postexercise recovery blood pressure in normotensive men (Pescatello et al., 1991). Furthermore, previous studies have shown no evidence for a prolonged blood pressure lowering effect from a single session of graded maximal cycling (Somers et al., 1991) or low to moderate intensity cycling (Brandao Rondon et al., 2002; Pescatello et al., 1991) when subjects continue their normal activities of daily living at home after the cessation of exercise until the following day.

The sustained postexercise hypotension needs to be interpreted with caution since the present study did not measure blood pressure once subjects had left the laboratory on day 1 until the next morning. Previous studies have shown a reduction in ambulatory systolic blood pressure following accumulated activity in prehypertensive and hypertensive individuals (Padilla et al., 2005). Similarly, studies have shown a
reduction in ambulatory systolic and diastolic blood pressure after a single bout of exercise lasting 30 minutes (Quinn, 2000) or 50 minutes (Wallace et al., 1999) in hypertensive individuals. It could be speculated that prehypertensive and hypertensive individuals may have a better baroreflex sensitivity and/or a better persisting effect on increased vasodilation in response to exercise compared with normotensive individuals. However, there are no data available at present to support this assertion.

The findings reported in Parts II and III of this chapter provide further support for the current physical activity recommendation that health benefits can accrue from both accumulated and continuous exercise (Pate et al., 1995). Since blood pressure often increases with age, regular physical activity may provide protection against the development of hypertension. Moreover, a randomised clinical trial has demonstrated that lifestyle-related accumulated activity is as effective in reducing systolic and diastolic blood pressure as structured exercise in normotensive middle-aged individuals (Dunn et al., 1997). This has important implications for the promotion of physical activity i.e. activities of daily living may be effective in maintaining normal blood pressure levels.

The studies reported here were all performed in the laboratory. Such environmental conditions may not apply to free-living situations. Nonetheless, the possibility that blood pressure may be lowered following the accumulation of 30 minutes of walking/running in 3 minute bouts may be attractive to individuals with low fitness levels. An interesting topic for future study would be to investigate whether the accumulation of exercise in short bouts over several weeks/months has an independent and additive effect on post-exercise hypotension.
In conclusion, the studies reported in this chapter demonstrate that the accumulation of exercise throughout a single day is effective in lowering systolic blood pressure on the following day in normotensive, healthy young men. Such effects were noted with as little as 30 minutes of walking accumulated in three-minute bouts. These findings have important implications for the prescription of exercise as a means to preventing/ameliorating hypertension.
CHAPTER 9
GENERAL DISCUSSION

The aim of this chapter is to integrate the findings from all experimental studies described in this thesis. To provide an overall picture from the experimental chapters 4, 5, 6, 7 and 8, a summary of the influence of accumulated exercise on cardiovascular disease risk factors is discussed first. Thereafter, the findings from Chapters 5 and 6 comparing postprandial triacylglycerol concentrations after accumulated versus continuous exercise are discussed. Then, the effects of exercise volume and timing on the magnitude of change in postprandial triacylglycerol concentrations are addressed (Chapters 4, 5, 6 and 7). This is followed by an examination of the effects of exercise intensity/duration on blood pressure (Chapter 8). High-sensitivity C-reactive protein and acylated ghrelin responses to short bouts of exercise (Chapter 7) are then discussed and this section ends with some recommendations for future research.

9.1 The effects of accumulating short bouts of exercise on cardiovascular disease risk factors

The data from this thesis have shown that accumulating short bouts of exercise reduces postprandial triacylglycerol concentrations and resting blood pressure in young men. These findings support the current physical activity guidelines (Pate et al., 1995; U.K. Department of Health, 2004; U.S. Department of Health and Human Services, 1996) and extend the guidelines by demonstrating that activity performed in bouts lasting less than 10 minutes can also be beneficial, at least in the case of postprandial triacylglycerol concentration and resting blood pressure. The findings on accumulated running and high-sensitivity C-reactive protein described in Chapter 7 have shown that
performing short bouts of running does not evoke the acute-phase inflammatory response observed in more prolonged/strenuous activity. These data imply that accumulating short bouts of activity may be an additional physical activity option for the public.

9.2 The effect of different patterns of exercise on postprandial triacylglycerol concentrations

A few studies have compared the effects of accumulated/intermittent exercise with a single bout of exercise on postprandial triacylglycerol concentrations (Altena et al., 2004; Gill et al., 1998; Murphy et al., 2000). These studies report that the reduction in postprandial triacylglycerol concentrations is comparable for accumulated versus continuous activity (Gill et al., 1998; Murphy et al., 2000) or even greater with accumulated versus continuous exercise (Altena et al., 2004). However, no study has been performed that addresses the effects of multiple bouts (lasting less than 10 minutes each bout) on postprandial triacylglycerol concentrations. The studies presented here investigated this issue in order to assess the impact of short duration of exercise on postprandial triacylglycerol concentrations. The results of the studies described in Chapters 5 and 6 demonstrate that accumulating 30 minutes of walking/running in very short (3 minute) bouts is equally as effective in reducing postprandial plasma triacylglycerol concentrations as one continuous 30 minute bout.

The reduction in postprandial triacylglycerol concentrations with both patterns of exercise reported here is consistent with the findings of previous studies employing young, active individuals (Gill et al., 1998) and middle-age, sedentary individuals (Murphy et al., 2000). However, in contrast to the present findings, Altena et al (2004)
reported that the magnitude of postprandial triacylglycerol concentration reduction was
greater after intermittent running (three, 10-minute bouts with 20 minutes rest between
each bout) than after the same amount of running performed in one continuous bout.
The discrepancy between the present findings and those of Altena et al (2004) may be
explained by a variety of factors. It has been shown that the magnitude of
catecholamine increase is positively related to the duration of exercise (Kjaer et al.,
1985), and endogenous and exogenous carbohydrate availability is known to affect
lipolytic rate by the presence of insulin (Campbell et al., 1992). Thus, differences in
exercise duration and food intake between the study of Altena et al (2004) and the
present study may explain the discrepant findings.

The study described in Chapter 5 attempted to evaluate the speculation that excess
post-exercise oxygen consumption may be one explanation for the lowering of
postprandial triacylglycerol concentration in the intermittent exercise trial, as proposed
by Altena et al (2004). In order to address this issue the present author measured
recovery oxygen uptake for 5 minutes after each bout of exercise in the accumulated
exercise trial, and these measurements were also made at equivalent time points in the
continuous exercise and control trials. The study found a significantly higher total
oxygen uptake in the accumulated exercise trial (38.2 ± 1.7 L) than in the continuous
exercise (20.1 ± 0.9 L) and control (16.8 ± 0.8 L) trials (Chapter 5). However, there
was a comparable reduction in postprandial triacylglycerol concentrations after both
accumulated and continuous exercise trials on the following day (Chapter 5),
indicating that excess post-exercise oxygen uptake was not the factor responsible for
the reduction in postprandial lipaemic responses. Excess post-exercise oxygen
consumption is short lived after short bouts of exercise and therefore the clearances are
possibly insufficient to influence postprandial triacylglycerol concentrations.

In summary, postprandial plasma triacylglycerol concentration is reduced to a similar extent when ten, three-minute bouts of walking or running are performed during the course of a day in comparison with one continuous 30-minute bout of walking or running in young men. These findings suggest that health benefits may accrue from the accumulation of very short bouts of physical activity and it may be unnecessary for physical activity guidelines for health to stipulate that physical activity be performed in minimum of 10 minute bouts.

9.3 The effect of different volumes of exercise on postprandial triacylglycerol concentrations

A review paper suggests that modest negative correlations exist between the volume of exercise performed and the magnitude of change in postprandial triacylglycerol concentrations i.e. a higher exercise volume are associated with greater reductions in postprandial triacylglycerol concentrations (Petitt and Cureton, 2003). This issue is addressed here by combining the findings of the studies described in Chapters 4, 5 and 6. It was felt appropriate to do this since the standardisation of physical activity, diet and alcohol consumption was identical in each study. The data demonstrate that there was no significant relationship between the volume of exercise performed and the magnitude of change in both total and incremental postprandial triacylglycerol concentrations (Figures 9.1 and 9.2) even after controlling for individual physical characteristics (Table 9.1). Thus, in the present studies exercise volume does not appear to be the key factor responsible for the reductions in postprandial triacylglycerol concentrations and other mechanisms may be involved.
Figure 9.1 Correlations between the magnitude of change in area under the curve values for total triacylglycerol concentration and exercise energy expenditure \((N = 44; n = 19, \text{Chapter } 4 (\bullet), n = 10, \text{Chapter } 5 (\triangle) \text{ and } n = 15, \text{Chapter } 6 (\blacksquare))\). Data were analysed using Pearson product-moment correlation coefficients. Note: the magnitude of change in total triacylglycerol concentration was calculated by subtracting the total area under the triacylglycerol concentration versus time curve on the control trial from the value obtained on the accumulated exercise trial.
Figure 9.2 Correlations between the magnitude of change in area under the curve values for incremental triacylglycerol concentration and exercise energy expenditure ($N = 44; n = 19$, Chapter 4 (○), $n = 10$, Chapter 5 (△) and $n = 15$, Chapter 6 (■)). Data were analysed using Pearson product-moment correlation coefficients. Note: the magnitude of change in incremental triacylglycerol concentration was calculated by subtracting the incremental area under the triacylglycerol concentration versus time curve on the control trial from the incremental value obtained on the accumulated exercise trial.
Table 9.1 Correlations between various subject characteristics and the magnitude of change in triacylglycerol (TAG) and systolic blood pressure (SBP)\(^1\) after accumulated exercise in

<table>
<thead>
<tr>
<th>Variable</th>
<th>Delta total TAG(^2)</th>
<th>Delta incremental TAG(^2)</th>
<th>Delta SBP(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.03</td>
<td>0.12</td>
<td>0.01</td>
</tr>
<tr>
<td>Weight</td>
<td>-0.19</td>
<td>-0.60</td>
<td>0.02</td>
</tr>
<tr>
<td>Body mass index</td>
<td>-0.23</td>
<td>-0.12</td>
<td>0.14</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>-0.18</td>
<td>0.18</td>
<td>0.15</td>
</tr>
<tr>
<td>(\dot{V}O_{\text{max}})</td>
<td>0.05</td>
<td>-0.08</td>
<td>-0.37</td>
</tr>
<tr>
<td>Initial SBP</td>
<td>-0.26</td>
<td>-0.06</td>
<td>-0.30</td>
</tr>
<tr>
<td>Partial correlation(^4)</td>
<td>0.08</td>
<td>-0.07</td>
<td>0.14</td>
</tr>
</tbody>
</table>

\(^1\) For TAG data; \(N = 44\). For SBP data; \(N = 25\).

Relationships between variables were evaluated using Pearson product-moment correlation coefficients. No significant correlations.

\(^2\) calculated by subtracting the total/incremental area under the concentration versus time curve in the control trial from the equivalent value obtained in the accumulated exercise trial.

\(^3\) calculated by subtracting baseline systolic blood pressure on day 1 of the accumulated exercise trial from baseline systolic blood pressure on day 2 of the accumulated exercise trial.

\(^4\) Controlling for all physical characteristics.
There was wide individual variation in the magnitude of change in total postprandial triacylglycerol concentrations within each study (Figure 9.1). In addition, 35 out of 44 individuals had a lower total postprandial triacylglycerol concentration after accumulated exercise compared with the control trial. Collectively, these findings show that there were so-called responders on one hand and non-responders on the other. This inter-individual variation is possibly explained by genetic factors. It has been suggested that apolipoprotein E plays a role in hepatic lipoprotein metabolism and uptake since this apolipoprotein has a strong affinity to bind with hepatic low-density lipoprotein receptors (Mahley, 1988). However, the role of apolipoproteins E varies depending on the phenotype, apolipoprotein E4 is thought to slow the postprandial catabolic pathway of triacylglycerol (Dart et al., 1997). However, a retrospective study has demonstrated that there was a similar reduction in the magnitude of change in exercise-induced postprandial triacylglycerol concentrations and individuals possessing different apolipoprotein E4 phenotypes (Gill et al., 2002). More research is therefore needed to investigate the interaction between genetic factors and postprandial triacylglycerol metabolism in response to exercise.

In relation to the inter-individual variation in the exercise-induced change in postprandial triacylglycerol concentrations, it is likely that there was a lowering of postprandial lipaemia in the early post-exercise period (i.e. day 1 of the trial). In the studies reported in Chapters 4, 5 and 6, blood samples were not collected during day 1. However, the study reported in Chapter 7 demonstrates that accumulating short bouts of exercise reduces postprandial triacylglycerol concentrations measured on the same day as test meals are consumed. Therefore, it could be argued that those who had no change in postprandial triacylglycerol concentrations on day 2 are not necessarily
non-responders, perhaps there was a greater reduction in postprandial triacylglycerol concentrations in these subjects on the day when accumulated exercise was performed. This may partly explain the lack of association between energy expenditure and plasma triacylglycerol lowering on day 2.

However, comparisons between the extent of reduction in postprandial lipaemia observed in Chapters 4, 5 and 6 should be interpreted with caution. Although the physical characteristics of subjects were similar between these studies, different subjects were employed in each study. Furthermore, although diet was standardised between trials in each study, it is possible that different evening meals with different carbohydrate content could influence the triacylglycerol response (Parks and Hellerstein, 2000). Thus, the findings are not directly comparable. Therefore, it is difficult to conclude that there was no dose-response between exercise volume and a reduction in postprandial triacylglycerol in the studies described in this thesis. Hence, direct comparisons of different volumes of accumulated activity within a single group of subjects are required to determine more clearly the influence of energy expenditure and postprandial lipaemia.

In summary, the volume of accumulated exercise does not appear to be related to the magnitude of reduction in postprandial triacylglycerol concentrations in the present studies. This is a surprising and intriguing finding and one that is worthy of further investigation. If exercise volume is not the primary factor responsible for reductions in postprandial triacylglycerol concentration after accumulated exercise it would be interesting to identify which factor is primary responsible. Unlike a dose-response relationship between exercise volume and a reduction in postprandial triacylglycerol.
concentrations observed in studies with a single bout of exercise (Petitt and Cureton, 2003), it could be the case that frequent increases in muscle blood flow through accumulating short bouts of exercise enhance exposure of triacylglycerol to muscle lipoprotein lipase and thus may be the primary factor responsible for a reduction of postprandial triacylglycerol concentrations regardless of exercise volume.

9.4 The effect of the timing of exercise on postprandial triacylglycerol concentrations
The studies described in Chapters 5 and 7 indicate that the magnitude of the reduction of total postprandial triacylglycerol concentrations tends to be greater ($P = 0.062$) following the accumulation of 30 minutes of running performed the day before postprandial testing (22% reduction in area under the curve values for total triacylglycerol concentration) (Chapter 5) than a similar total volume of running performed the same day as postprandial testing (10% reduction in area under the curve values for total triacylglycerol concentration) (Chapter 7). These quantitively different lipaemic responses to the timing of exercise may be explained partly by the activity of skeletal muscle lipoprotein lipase since skeletal muscle lipoprotein lipase activity is maximised more than 8 hours after exercise (Seip et al., 1997). This is perhaps why a majority of studies involving exercise the day before the consumption of the test meals have observed an attenuation in postprandial triacylglycerol concentration whereas some studies using a one-day model (exercise and postprandial testing on the same day) have not observed a reduction in postprandial lipaemia with exercise (Petridou et al., 2004; Pfeiffer et al., 2005; Zhang et al., 1998).

With regard to the effects of the timing of exercise on postprandial lipaemia, in
Chapters 5 and 6 the author employed a single bout of 30 minutes of brisk walking or running completed in the afternoon at 15:30. There were two reasons for this. Firstly, the author wanted to standardise the time interval between the cessation of exercise and the consumption of the first test meal (on the following day) in both the accumulated exercise and continuous exercise trials. Secondly, the interval of 17 hours (i.e. 15:30 to 08:30) was designed to provide adequate time for maximising muscle lipoprotein lipase activity. A previous study has demonstrated that exercise-induced increases in muscle lipoprotein lipase mass are transient and such effects may be dissipated 20 hours after a single session of moderate intensity exercise (85-90 minutes at 65% of maximal oxygen uptake) (Seip et al., 1997). Therefore, if a single bout of 30 minutes of brisk walking or running was performed at 9:30 on the morning of day 1, a reduction in postprandial triacylglycerol concentrations may not be seen on the following morning.

It is worth mentioning, however, that the studies which have failed to detect exercise-induced postprandial triacylglycerol lowering effects (Petridou et al., 2004; Pfeiffer et al., 2005; Zhang et al., 1998) must be interpreted with caution. These studies have not corrected for free glycerol concentrations on the exercise trial – hence triacylglycerol concentrations may be overestimated for the exercise trial since in this state free glycerol concentration is increased, particularly following a prolonged bout of exercise (Hardman and Aldred, 1995; Schlierf et al., 1987). It is possible that the failure to correct for free glycerol concentrations may have led to over-estimations of postprandial triacylglycerol concentration on the exercise trial in these studies. Although free glycerol was not corrected for in the study reported in Chapter 7 here, it is reasonable to speculate that free glycerol concentration would not be markedly
increased during each bout of running since the duration was only 5 minutes. Moreover, blood samples were taken 85 minutes before each run which may have provided enough time for glycerol volume to return to baseline concentrations as has been shown in a previous study which involved a single bout of exercise (Schlierf et al., 1987). This methodological issue should be kept in mind when evaluating studies involving concurrent exercise and postprandial testing. Nonetheless, the findings from Chapter 7 demonstrate that accumulated exercise is effective in reducing postprandial lipaemia even when postprandial testing is performed concurrently with exercise.

In addition, the study reported in Chapter 7 adds to the understanding of mechanisms regarding an early effect of accumulated exercise on postprandial triacylglycerol concentrations. Since postprandial plasma triacylglycerol concentrations were reduced, as early as half way through the exercise trial compared with the control trial, this suggests that muscle lipoprotein lipase activity may increase soon after the first two/three bouts of exercise. It is also possible that accumulated exercise increased the exposure of triacylglycerol to muscle lipoprotein lipase through an increase in muscle blood flow for a prolonged period throughout the day. If this is the case, further research is required to investigate whether muscle blood flow and muscle lipoprotein lipase activity are elevated when accumulating short bouts of exercise compared with a single bout of exercise matched for total energy expenditure.

9.5 The effect of exercise volume on post-exercise hypotension

Although there are inconsistent findings regarding the magnitude of post-exercise hypotension (Halliwill, 2001), several intervention studies have shown that the magnitude of post-exercise hypotension does not differ greatly with different exercise
intensities (MacDonald et al., 1999) and durations (MacDonald et al., 2000).

Inter-study comparisons are difficult between the present three studies reported in this thesis because different blood pressure measurement equipment was used for the first study (Part I) compared with the second and third studies (Parts II and III). However, baseline systolic blood pressure measured on day 1 in the accumulated exercise trial could be compared with baseline systolic blood pressure measured on day 2 in the accumulated running and walking trials described in Parts II and III since the study designs were identical. The data demonstrate that there was no significant relationship between the energy expenditure in accumulated exercise and the magnitude of change in post-exercise systolic blood pressure (Figure 9.3) even after controlling for individual physical characteristics and initial blood pressure (Table 9.1). These data provide evidence of beneficial effects of accumulating short bouts of exercise throughout the day in normotensive individuals. It is worth noting that both studies used an exercise duration of 3-minutes per bout which is considered an attractive strategy for many individuals who wish to incorporate exercise into their daily activities. Moreover, the magnitude of the reduction in systolic blood pressure (10 mm Hg in Part II and 11 mm Hg in Part III) the day after accumulated activity is large and could be clinically important for those with pre-hypertension and/or hypertension. Obviously it is important to replicate these findings in such groups prior to recommending such activity patterns.
Figure 9.3 Correlations between the magnitude of change in systolic blood pressure (SBP) and exercise energy expenditure ($N = 25; n = 10$, Chapter 5 (△) and $n = 15$, Chapter 6 (■)). Data were analysed using Pearson product-moment correlation coefficients. Note: the magnitude change in SBP was calculated using baseline SBP measured on day 2 subtracted from baseline SBP measured on day 1 in the accumulated exercise trials.

In summary, the magnitude of change post-exercise hypotension is not related to the energy expenditure accumulated during exercise in the present studies described in Chapter 8 Parts II and III. With respect to the short exercise durations used in the present studies, the findings suggest potential cardioprotective effects if these interventions are found to be effective in middle-aged and older aged groups. Further studies are needed to investigate whether lifestyle activities are effective in reducing resting blood pressure in groups of varied age and health status.
9.6 The effects of accumulated exercise on high-sensitivity C-reactive protein and acylated ghrelin concentrations

The findings described in Chapter 7 show that accumulating short bouts of running does not increase serum high-sensitivity C-reactive protein concentrations. This suggests that the acute-phase inflammatory response which typically occurs after prolonged strenuous exercise (Fallon, 2001; Siegel et al., 2001; Taylor et al., 1987; Weight et al., 1991) does not occur after the accumulation of moderate-intensity exercise. However, serum high-sensitivity C-reactive protein was only measured at two time-points, i.e. baseline and the end of the day in the exercise and control trials. C-reactive protein binds to foreign substances resulting from tissue injury or infection and it reacts fast (biological half-life of 19 hours) (Ledue and Rifai, 2003), thus additional measurements of high-sensitivity C-reactive protein after each bout of exercise would provide a more comprehensive assessment of the influence of accumulated activity on C-reactive protein concentrations.

A strength of the study described in Chapter 7 is that serum high-sensitivity C-reactive protein concentration was measured using an enzyme-linked immuno sorbent assay which had a high sensitivity (detection limit = 0.1 mg/L). From a clinical point of view, reliable and accurate measurement of high-sensitivity C-reactive protein is necessary for risk stratification for cardiovascular disease. Since regular exercise has anti-inflammatory effects (Petersen and Pedersen, 2005) and long-term exercise training lowers C-reactive protein concentrations (Kondo et al., 2006; Lakka et al., 2005; Mattusch et al., 2000; Smith et al., 1999; Tisi et al., 1997), assessment of the influence of weeks/months of accumulated physical activity on high-sensitivity C-reactive protein may be a fruitful area for future research.
The data from the study described in Chapter 7 indicated that plasma acylated ghrelin concentration did not change during the accumulation of short bouts of exercise. The study also indicated that plasma acylated ghrelin concentration was not suppressed after eating. If ghrelin concentration is mediated by the current energy status of individuals as is suspected (Cummings et al., 2001), it is difficult to understand why there was no alteration in plasma acylated ghrelin concentration observed in this study. However, it is possible that the energy deficit created by accumulated exercise was too small to elicit change. Support for this notion comes from the finding that a 3-week integrated body weight reduction programme (energy-restricted diet, nutritional education, psychological counselling and exercise training) did not increase fasting serum total ghrelin concentrations (Morpurgo et al., 2003) whereas fasting serum total ghrelin concentrations were increased after a 3-month energy deficit programme involving diet and exercise (Leidy et al., 2004). Therefore, a large and prolonged energy deficit may be required to alter plasma ghrelin concentrations.

The failure to suppress postprandial ghrelin levels may be due in part to the macronutrient composition of meal provided in the study described in Chapter 7. Plasma acylated ghrelin may be more sensitive to carbohydrate-rich meals than fat-rich meals such as that used in the present study, as suggested by a previous study (Tentolouris et al., 2004). In terms of acylated ghrelin metabolism response to exercise, clinical benefits are unknown to date. The present author’s research group has demonstrated that plasma acylated ghrelin concentrations are reduced during and immediately after a 1 hour bout of high-intensity running. Appetite suppression also occurred in this study which was conducted in young healthy men (unpublished data). It would be interesting to investigate whether acylated ghrelin responds in the same
way to an exercise induced energy deficit as to a dietary induced energy deficit. In addition, further research is required to assess whether acylated ghrelin responses to exercise are similar in obese versus non-obese individuals.

9.7 Conclusion
In conclusion, the studies described in this thesis show that accumulating short bouts of exercise throughout a single day lowers postprandial triacylglycerol concentrations and resting blood pressure without altering high-sensitivity C-reactive protein, indicating that such short bouts of accumulated exercise may beneficially influence cardiovascular disease risk. Furthermore, another key finding of the studies reported here is that similar health benefits in terms of postprandial triacylglycerol concentrations and resting blood pressure may be obtained through accumulated versus continuous activity. These findings imply that the duration of exercise may not be important for some aspects of disease prevention and this is important since some individuals may prefer to accumulate activity in short bouts throughout the day rather than perform all of their exercise in one go. In particular, this may apply to people with low fitness levels and/or busy work schedules.

9.8 Recommendations for future research
1. It would be interesting to examine whether exercise bouts of shorter duration to those studied here are effective in reducing postprandial lipaemia and/or resting systolic/diastolic blood pressure.

2. Studies are required to determine if the lowering of postprandial lipaemia and resting blood pressure demonstrated here also occur in older age groups and in those with hypertriacylglycerolaemia and hypertension.
3. Studies are required to confirm the findings reported here in a more realistic setting i.e. outside of the laboratory.

4. Additional research would be useful to determine the influence of accumulated activity bouts performed over several days/weeks on postprandial lipaemia and resting blood pressure to assess whether these are chronic effects which are independent of the acute effects of exercise.

5. Further research is necessary to evaluate more comprehensively the responses of acylated ghrelin and C-reactive protein to accumulated activity.
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APPENDICES

Appendix A: Information for Participants

Appendix B: Statements of Informed Consent

Appendix C: Health Screen for Study Volunteers

Appendix D: Physical Activity Questionnaire
Appendix A: Information for Participants

LOUGHBOROUGH UNIVERSITY
SCHOOL OF SPORT AND EXERCISE SCIENCES

The effect of intermittent bouts of aerobic exercise on fat metabolism and blood pressure

INVESTIGATORS

Masashi Miyashita and David Stensel

BACKGROUND

Cardiovascular disease (CVD) is a major cause of death and disability in the United Kingdom and in many other developed countries. One of the major risk factors for CVD is high blood pressure (hypertension). Another risk factor is an elevation in the level of fat (triacylglycerol) in the blood. Previous research has shown that a single session of aerobic exercise is effective in lowering blood pressure for several hours following exercise. Studies have also demonstrated that aerobic exercise performed prior to a meal is effective in reducing the rise in blood fat levels which typically occurs after eating. Therefore, these are two possible mechanisms by which regular exercise may reduce the risk of CVD. However, the effects of exercise on blood pressure and blood fat levels have been investigated using continuous bouts of exercise. Whether short-intermittent bouts of exercise are effective in lowering blood pressure and blood fat remains to be determined. It is important to answer this question because many people may find it easier to incorporate intermittent exercise into their lives. Therefore, the purpose of the this study is to examine the influence of repeated short bouts of aerobic exercise (running) on blood fat levels and blood pressure. After some preliminary tests the study will involve two main trials each conducted over two days. One of these trials will be an exercise trial and the other will be a control trial. Details of the preliminary tests and the main trials are given below.
PRELIMINARY PROCEDURES

Before enrolling on the study you will be asked to attend the laboratory for a preparatory session during which we will:
• explain the objectives of the study and its requirements;
• measure your blood pressure;
• ask you to complete a confidential questionnaire regarding your health;
• familiarise you with the testing procedures and equipment;
• familiarise you with dietary recording;
• answer any questions you may have.

Following this you will be asked to return to the laboratory on separate days to complete two exercise tests as follows:

Submaximal treadmill test: You will run on a treadmill for 16 minutes. The speed of the treadmill will be increased every four minutes. You will periodically be asked to breathe through a mouthpiece while wearing nose clips during the run.

Maximum oxygen uptake test: You will run on a treadmill. The incline of the treadmill will be increased every three minutes. You will periodically be asked to breathe through a mouthpiece while wearing nose clips during the run. You will run until volitional fatigue. This will take between 10 and 12 minutes.

MAIN TRIALS

There will be two main trials and each one will be conducted over two-days.

Exercise trial – day one: you will report to the laboratory at 9:00 am having eaten breakfast. You will spend the day (9:00 am to 4:00 pm) in the laboratory. You will perform 10 to 15 six-minute treadmill runs over the course of the day. These runs will be of moderate intensity (70% of maximum oxygen uptake). Your blood pressure will be measured at various intervals during the day and samples of your expired air will also be collected.

Exercise trial – day two: you will report to the laboratory at 8:00 am having fasted for 10 hours i.e. since 10:00 pm the previous day (you can drink water during this period). A cannula (small plastic tube) will be inserted into a vein in your arm and a small blood sample will be collected (for those of you who have given blood the procedure is the same). Samples
of you expired air will then be collected over a 15-20 minute period while you rest on a bed. You will then be given a breakfast comprising of a cheese sandwich (bread, butter, cheese and mayonnaise), potato crisps and a milk shake. You will remain in the laboratory until 4:00 pm. During this time you will rest quietly (reading, writing, watching TV, listening to music). Blood samples will be collected throughout the day and further samples of expired air will be collected. You will also be given lunch at around 12:00 pm. This will be a repeat of the meal you consumed at breakfast.

Control trial – day one: you will report to the laboratory at 9:00 am having eaten breakfast. You will spend the day (9:00 am to 4:00 pm) in the laboratory. You will rest throughout the day (reading, writing, watching TV, listening to music). Your blood pressure will be measured at various intervals during the day and samples of your expired air will be collected.

Control trial – day two: this will be identical to day two of the exercise trial. No more than 200 ml of blood will be removed in total over the two trials (much less than the amount collected in one routine blood donation).

PREPARATION FOR THE TESTS

- **Recording your diet:** the day before day one of your first main trial you will be asked to weigh and record everything you eat and drink. You will then consume identical amounts of the same food and drink on the day before the start of your second main trial. You will also be asked to weigh everything you eat and drink on day one of your first main trial and to replicate this during your second main trial. This is very important to the control of the experiment and we will discuss it with you prior to the main trials. No alcohol should be consumed on the days when you are recording your diet.

- **Controlling physical activity:** this is crucial to the success of the experiment and you should remain inactive on the day before the start of each main trial and throughout the main trials (other than the exercise performed as part of the experiment).

- **Ten-hour overnight fast:** you will finish eating by 10 o’clock on the evenings before the second day of each main trial. You may continue to drink water after this time. You will report to the laboratory at 8 o’clock the following morning without taking breakfast.
• **Traveling to the laboratory:** if you live within 400 m of the laboratory you should walk in slowly on the morning of the second day of each main trial. Please do not run or cycle. If you live more than 400 m from the laboratory then you should drive in. If you do not have access to a car please inform us and we will arrange for you to be collected.

• **Showering:** it is important that you do not shower in the mornings prior the second day of each main trial since this may alter your resting energy expenditure (resting metabolic rate). Therefore, we recommend that you shower the night before the second day of each main trial.

• **Keeping warm:** it is very important that you keep warm on the mornings of the second day of each main trial. Collecting blood may be difficult if you are cold. Please wear a coat and sweater on your way to the laboratory and keep your sweater on when you reach the laboratory.

**HOW MUCH TIME WILL THE TESTS TAKE?**

- Preliminary visit: approximately 45 minutes
- Submaximal treadmill test: approximately 30 minutes
- Maximum oxygen uptake test: about 30 minutes.
- Main trials: four days in total – two days for each trial

**POSSIBLE RISKS AND DISCOMFORTS**

The maximum oxygen uptake test will cause physical exhaustion but you should recover within a few minutes. Blood sampling may cause minor bruising, an inflammation of the vein or haematoma (a small accumulation of blood under the skin). There is also an exceedingly small risk of a tiny piece of plastic or an air bubble entering the bloodstream if the cannula is incorrectly placed. Good practice, however, minimises this risk and only experienced and trained staff will deal with blood sampling.
BENEFITS OF THE STUDY

The findings will be published in the scientific literature so that understanding of the way in which exercise influences the risk of heart disease can be increased. We will provide you with feedback about the overall findings as well as your own results and will be happy to explain results and discuss the implications with you.

CONFIDENTIALITY

Although information will be stored on computer, each subject will be entered as a number rather than by name, in accordance with the Data Protection Act.

Please feel free to ask any questions, at any stage. Contact information for the staff involved in the testing is as follows:

Mr Masashi Miyashita, M.Miyashita@lboro.ac.uk, tel: 01509 226351

Dr David Stensel, D.J.Stensel@lboro.ac.uk, tel: 01509 226344
Appendix B: Statements of Informed Consent

LOUGHBOROUGH UNIVERSITY
SCHOOL OF SPORT AND EXERCISE SCIENCES

Project title: The effect of intermittent bouts of aerobic exercise on fat metabolism and blood pressure

I understand that my permission to take part in these tests is voluntary and that I am free to withdraw at any point and without explanation.

I have read the information regarding these tests and had the opportunity to ask questions of the testers. I understand the procedures involved and the risks have been explained to me. I give my consent to participate in this study.

Signature of subject:........................................ Date:....................................

Signature of witness:..........................................
Appendix C: Health Screen for Study Volunteers

It is important that volunteers participating in research studies are currently in good health and have had no significant medical problems in the past. This is to ensure (i) their own continuing well-being and (ii) to avoid the possibility of individual health issues confounding study outcomes.

Please complete this brief questionnaire to confirm fitness to participate:

1. **At present, do you have any health problem for which you are:**
   - (a) on medication, prescribed or otherwise ........................................ Yes □ No □
   - (b) seeing your general practitioner .................................................. Yes □ No □
   - (c) on a hospital waiting list ............................................................. Yes □ No □

2. **In the past two years, have you had any illness which required you to:**
   - (a) consult your GP ........................................................................ Yes □ No □
   - (b) attend a hospital outpatient department ...................................... Yes □ No □
   - (c) be admitted to hospital ............................................................... Yes □ No □

3. **Have you ever** had any of the following:
   - (a) Convulsions/epilepsy ................................................................. Yes □ No □
   - (b) Asthma ..................................................................................... Yes □ No □
   - (c) Eczema ..................................................................................... Yes □ No □
   - (d) Diabetes .................................................................................... Yes □ No □
   - (e) A blood disorder ........................................................................ Yes □ No □
   - (f) Head injury ................................................................................ Yes □ No □
   - (g) Digestive problems ..................................................................... Yes □ No □
   - (h) Heart problems .......................................................................... Yes □ No □
   - (i) Problems with bones or joints .................................................... Yes □ No □
   - (j) Disturbance of balance/co-ordination ......................................... Yes □ No □
   - (k) Numbness in hands or feet .......................................................... Yes □ No □
(l) Disturbance of vision .......................................................... Yes ☐  No ☐
(m) Ear/hearing problems .................................................. Yes ☐  No ☐
(n) Thyroid problems .................................................... Yes ☐  No ☐
(o) Kidney or liver problems ........................................ Yes ☐  No ☐
(p) Allergy to nuts ...................................................... Yes ☐  No ☐

4. Has any, otherwise healthy, member of your family under the age of 35 died suddenly during or soon after exercise? ............. Yes ☐  No ☐

5. Have you ever, been a regular smoker? ................................. Yes ☐  No ☐

If YES to any question, please describe briefly if you wish (eg to confirm problem was/is short-lived, insignificant or well controlled.)
Appendix D: Physical Activity Questionnaire

Name

We are interested in finding out about your physical activity levels that you undertake as part of your everyday lives. The questions will ask you about the time you spent being physical active in the last 3 months.

*Note.* Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

1. Do you currently undertake exercise (aerobic exercise and/or resistance exercise)?

☐ YES

☐ NO

If your answer is YES, please answer the following questions.

2. During the last 3 months, on how many days did you undertake vigorous physical activities and how much time in total did you usually spend (including accumulating exercise and/or continuous exercise) on one of those days doing vigorous physical activities?

_______ days per week

_______ minutes per day

☐ NO vigorous physical activity
3. During the last 3 months, on how many days did you undertake moderate physical activities and how much time in total did you usually spend (including accumulating exercise and/or continuous exercise) on one of those days doing moderate physical activities?

_____ days per week

_____ minutes per day

☐ NO moderate physical activity