The Effect of Exercise on Lipid and Lipoprotein Metabolism in Adolescents and Young Adults

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Abstract

The studies described in this thesis were undertaken to examine the effects of exercise, particularly intermittent games activity, on fasting lipid and lipoprotein concentrations and postprandial lipaemia in adolescents and young adults.

In order to be confident of the measurements made in the experimental studies described in this thesis it was necessary to investigate the reliability and criterion validity of some of the techniques used in these studies. Therefore preliminary work was undertaken which compared the use of two different analysers (Reflotron and Cobas Mira Plus) to determine postprandial lipoprotein concentrations in venous and capillary blood. Triacylglycerol (TAG), glucose and total cholesterol concentrations measured from capillary whole blood using the Reflotron were on average 2% higher and 5 and 13.4% lower respectively, than those measured in venous plasma using the Cobas Mira Plus. High density lipoprotein (HDL) cholesterol concentrations measured from capillary plasma using the Reflotron were on average 14.7% lower than those measured in venous plasma using the Cobas Mira Plus. As 50% of the samples collected for TAG analysis were below the measurement range of the Reflotron analyser (0.80 mmol l^{-1}) it was decided that it was not a suitable analyser with which to measure TAG concentrations in the third study described in this thesis. In Chapters 5 and 6 the Loughborough Intermittent Shuttle Test (LIST) was used as a mode of exercise and it was necessary to have a portable method of determining oxygen uptake. Therefore, oxygen uptake measurements made using Douglas bags, carried on the back via a rucksack or connected to a rack were compared. The mean coefficient of variation obtained for oxygen uptake measurements when the two different methods were compared was 2.0 ± 1.7%, the Pearson correlation coefficient was 0.967, and the bias and limits of agreement were 0.05 and –2.33 – 2 43 (ml $kg^{-1}.min^{-1}$). These values were similar to those seen when two measurements, made using consecutive Douglas bags on a rack, were compared It was decided based on these findings that using the Douglas bag mounted on a rucksack was an adequate method of establishing oxygen uptake during the LIST protocol

The aim of the first study described in this thesis was to examine the association between cardiorespiratory fitness (as indicated by peak $\dot{V}O_2$ and percentage peak VO_2 at a blood lactate concentration of 2.5 mmol l^{-1}) and blood lipid and lipoproteins in adolescents. It was hypothesised that participants with higher cardiorespiratory levels would have more favourable blood lipid-lipoprotein profiles. Seventy-seven adolescent males (age (years)· 14.5 ± 0.2; body mass (kg): 58.0 ± 1 5; peak treadmill running $\dot{V}O_2$ (ml $kg^{-1}.min^{-1}$): 55.8 ± 0.8) and 62 adolescent females (age (years)· 14.2 ± 0.2; body mass (kg): 53.9 ± 1.3; peak treadmill running VO_2 (ml.$kg^{-1}.min^{-1}$): 44.3 ± 0 6) participated in this study Peak VO_2, sex and waist circumference were found to be important predictors of the natural logged HDL cholesterol (lnHDL cholesterol) and the total cholesterol/HDL cholesterol ratio in a group of adolescents These three predictor variables explained 17.1% of the variation in lnHDL cholesterol concentrations and 12.6% of the variation in the total cholesterol/HDL cholesterol ratio Significant correlations were also found between peak $\dot{V}O_2$ and the total cholesterol/HDL cholesterol ratio in both males and females (r = 0 247, P = 0 012; r = 0.315, P = 0.047 respectively) The results of this study indicate that for adolescents, peak $\dot{V}O_2$, sex and waist circumference may be important determinants of blood lipid-lipoprotein profile.

The second study described in this thesis examined the influence of intermittent games activity on postprandial lipaemia in young adult males. It was hypothesised that if the energy expended during a bout of such exercise was sufficiently large postprandial lipaemia would be reduced. Twelve male volunteers (age (years): 21.1 ± 0.4; body mass (kg)· 76.9 ± 1 8; peak treadmill walking VO_2 (ml kg^{-1} min^{-1})·53.0 ± 1.5) completed three

main trials, a minimum of 6 days apart, in a balanced cross-over design. In the Rest trial participants rested on day one and completed an oral fat tolerance test on day two In the Walk exercise and LIST exercise trials participants completed four blocks (approximately 15 min) of uphill treadmill walking or intermittent running respectively on day one, and the oral fat tolerance test on day two. Lactate concentrations and oxygen uptake measurements from the exercise trials suggested the LIST protocol elicited a higher exercise intensity (Walk vs LIST: 2.4 ± 0.3 vs 4.3 ± 0.6 [lactate, mmol.l^{-1}]; 61.6 ± 1.0 vs 71.8 ± 1.6 [%peak $\dot{V}O_2$]) The areas under the TAG versus time curve were 9.85 ± 0.77, 8 02 ± 0.85 and 7.41 ± 0.61 mmol l^{-1}.6 h in the Rest, Walk exercise and LIST exercise trials respectively (main effect trial, P = 0.001) Compared with Rest these represented reductions of 25 (LIST, P = 0.001) and 19% (Walk, P = 0.064). The findings of this study indicated that games activity could reduce postprandial lipaemia and therefore was an appropriate mode of exercise with which to meet recommended activity guidelines.

The process leading to coronary atherosclerosis is initiated in childhood (McGill *et al.*, 2000) and so reducing postprandial lipaemia, even in young people, could potentially slow atherogenic progression However, as the postprandial lipid responses of adolescents to exercise have not been investigated, and as adolescents have been shown to respond differently to exercise than adults (Boisseau & Delamarche, 2000), any benefits of exercise on lipid clearance from the blood in such a group is yet to be established. Therefore, the third and final study described in this thesis examined the influence of two different modes of exercise (Walk, LIST) on postprandial lipaemia in adolescent boys. Two groups of adolescent boys performed two main trials, a Rest trial and either a Walk exercise or LIST exercise trial a minimum of 7 days apart, in a randomised order (Walk group: n = 10, 15.3 ± 0.1 years; 63 4 ± 1.1 kg; 44 8 ± 3.4 ml.kg^{-1} min^{-1}; LIST group: n = 9; 15 4 ± 0.1 years; 59.8 ± 1.0 kg; 51.1 ± 2.5 ml.kg^{-1}.min^{-1}). In the Rest trial the boys took no exercise on day one and completed an oral fat tolerance test on day two. In the Walk exercise and LIST exercise trials the boys completed four blocks (approximately 15 min) of uphill treadmill walking or intermittent running respectively on day one, and the oral fat tolerance test on day two. The areas under the TAG versus time curve were 5.60 ± 0.51 and 4.82 ± 0.54 mmol.l^{-1}.6h in the Rest, Walk exercise trials respectively (main effect trial, P = 0.062); and 6.21 ± 0.78 and 4.60 ± 0.52 mmol.l^{-1}.6h in the Rest, LIST exercise trials respectively (main effect trial, P = 0.006). Exercise reduced the area under the TAG versus time curve by 14% (Rest, Walk exercise boys, main effect trial, P = 0 064) and 26% (Rest, LIST exercise boys; main effect trial, P = 0 006). The findings of this study indicated that games activity significantly reduced postprandial lipaemia in adolescent boys.

Clearly the studies described above show that exercise, particularly games activity, can influence lipid and lipoprotein metabolism in adolescents and young adults. They also provide evidence that it is important for adolescents and young adults to lead an active lifestyle, and that games activity provides an appropriate mode of exercise with which to meet recommended activity guidelines. The current physical activity guidelines suggest adults should perform at least 30 min of moderate intensity activity on most, but preferably all, days of the week (American College of Sports Medicine, 1998). It has been recommended that young people should perform at least 1 h of moderate activity per day (Biddle *et al.*, 1998).

Keywords: Postprandial lipaemia, adolescents, intermittent games activity, lipid-lipoprotein metabolism

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Preface

Unless otherwise indicated by reference to published literature or acknowledgement, the work contained herein is that of the author and has not been previously submitted for another degree to this or any other University.

Some of the results of the study presented in Chapter 4 of this thesis have been published as follows:

Published Communications:

Barrett LA, Morris JG, Stensel DJ, Nevill AM & Nevill ME (2004) Relationship between cardiorespiratory fitness and blood lipid profile in adolescent children. *Journal of Sports Sciences* 22, 272 - 273.

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Chapter 1: Introduction

Coronary heart disease (CHD) is the major cause of adult death in industrialized nations. It causes over 117,000 deaths a year in the UK: around one in five deaths in men and one in six deaths in women. This compares to around 34,000 a year from lung cancer, 16,000 deaths from colo-rectal cancer and 13,000 deaths from breast cancer. Although mortality from CHD is falling rapidly, morbidity from CHD and other heart disease is rising. CHD costs the health care system in the UK about £1,750 million a year and the UK economy about £5,300 million because of work days lost due to death, illness and informal care of people with the disease. In total CHD costs the UK economy about £7,055 million a year (British Heart Foundation, 2004).

Epidemiological studies have identified several risk factors for CHD including an adverse lipid profile, obesity, hypertension and cigarette smoking (Castelli, 1984). In addition, there is increasing evidence to suggest that physical inactivity or lack of exercise is also another important risk factor. The first study to suggest that increasing physical activity was a deterrent to the development of CHD was published in 1953 by Jeremy Morris and colleagues (1953). In their study they examined transport workers in London and found that sedentary bus drivers had a greater incidence of CHD than the more active conductors on double-decker buses. Since this seminal study, numerous epidemiological studies comparing groups of physically inactive with active subjects have been published. These studies have been reviewed and it has been concluded that physical activity is inversely and causally related to the incidence of CHD (Powell *et al.*, 1987; Berlin & Colditz, 1990).

There is growing evidence that the process leading to coronary atherosclerosis, which becomes clinically manifest as CHD in adult life, is initiated during childhood (McGill, 1984). Atherosclerosis is a disease of the muscular arteries that is characterised by the development, over decades, of fibrous and fatty lesions on the arterial intima. Atherosclerosis begins in childhood as deposits of cholesterol and its ester in the intima of large muscular arteries. Initially these lipid deposits are known as fatty streaks. Autopsy examinations reveal that virtually all children exhibit fatty streaks in the intima of the aorta, by the age of 3 years. Not all fatty streaks develop

into raised lesions (fibrous plaques and the other advanced lesions of atherosclerosis). There is histological evidence that fatty streaks can form, dissolve and reform (Stary, 2000). However at some sites in the coronary arteries fatty streaks thicken by continuous, or intermittent, accumulation of lipid and connective tissue to form fibrous plaques or atheroma. Over years, these atheroma gradually increase in size and can undergo calcification and vascularisation. Such advanced atherosclerotic lesions can occlude arteries, reduce blood supply to the heart and produce severe clinical symptoms (McGill *et al.*, 2000) (see Figure 1.1).

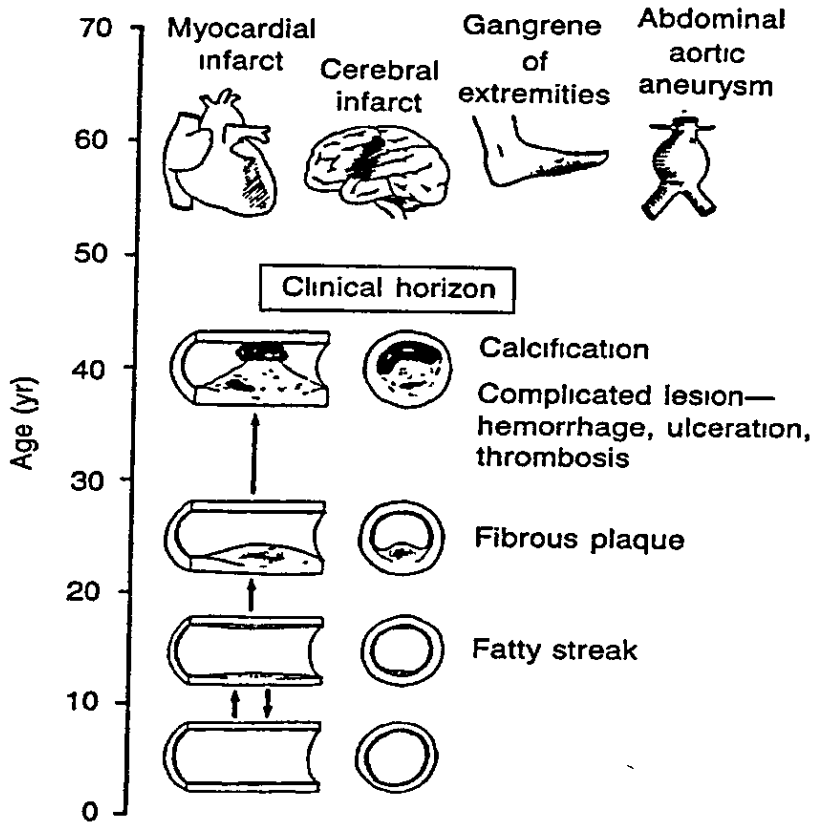

Figure 1.1 Represents the progression of atherosclerosis from fatty streak to a clinical horizon where the ensuing ischaemia leads to organ damage and symptoms develop. Reproduced from McGill *et al*, (2000)

Observations from autopsy studies by the Bogalusa Heart Study and the multi centre Pathobiological Determinants of Atherosclerosis in Youth study have documented a strong relationship between coronary atherosclerosis and CHD risk factors in young people (Berenson *et al.*, 1998). It has also been established that a significant number

of young people display at least one risk factor for CHD (Berenson *et al.*, 1980; Boreham *et al*, 1993) and that these risk factors can track into adulthood (Nicklas *et al.*, 2002). A recent study, which sought to provide estimates of overweight and obesity prevalence in English children and adolescents found that the frequency of overweight ranged from 22% at age 6 years to 31% at age 15 years and that of obesity ranged from 10% at age 6 years to 17% at age 15 years (Reilly & Dorosty, 1999). In addition the first cases of type II diabetes in white obese adolescents have been reported in the UK (Drake *et al.*, 2002). Type II diabetes is also being reported in children from the United States, Canada, Japan, Hong Kong, Australia, New Zealand, Libya and Bangladesh (American Diabetes Association, 2000). This type of diabetes had previously only been prevalent in adults; usually occurring in overweight and obese adults aged 40 and above it and as a result has sometimes been termed maturity-onset diabetes. However, the prevalence of the disease is rising in North America, and its incidence has almost doubled in Japan between 1976 – 1980 and 1991 – 1995 from 7.3 to 13.9 per 100,000 in junior high school children (Fagot-Campagna *et al.*, 2001). A recent study has demonstrated that hyperinsulinaemia (seen in people with type II diabetes) is an independent predictor of ischaemic heart disease after controlling for other CHD risk factors (Deprés *et al.*, 1996). There is substantial evidence that physical activity can be used as a means of enhancing insulin sensitivity and in so doing reduce hyperinsulinaemia (Borghouts & Keizer, 1999). It is therefore logical that the prevention of CHD should begin in childhood by attempting to control the modifiable risk factors for this disease: an adverse lipid profile, obesity, hypertension and hyperinsulinaemia.

Children and adolescents are not mini adults. As they grow and mature their physiological responses to physical activity and exercise vary as they progress through childhood and adolescence into adulthood (for review see Boisseau & Delamarche, (2000)). It has been suggested that children in contrast to adults may be "metabolic nonspecialists", that is children who possess a high peak $\dot{V}O_2$ and who can as a result perform well in endurance type exercise may also perform well in sprint type exercise. This theory is still a matter of debate and requires further investigation (Rowland, 2002). Consequently, the responses of children and adolescents to exercise may differ from those of adults. Exercise has been shown to have a beneficial influence on the lipid and lipoprotein profile of adults (Kraus et al., 2002), however the evidence in children and adolescents is limited and therefore requires further investigation. As we

spend most of day in the postprandial state, it is important not to focus solely on fasting lipid and lipoprotein concentrations. There is evidence that postprandial lipoproteins and their remnants may be directly atherogenic (Zilversmit, 1979) and that exercise can reduce postprandial lipaemia in adults (Hardman, 1998). As the process leading to coronary atherosclerosis begins during childhood (as evident by the presence of fatty streaks and raised lesions) (McGill, 1984), reducing postprandial lipaemia even in young people could potentially slow this atherogenic progression. Therefore, it is important to investigate what effect exercise has on postprandial lipaemia in children especially as their responses may differ from those of adults. This data could then be used in the development of physical activity guidelines for children and adolescents as research data to support the current guidelines are lacking. To date the majority of studies examining the effect of exercise on postprandial lipaemia have used continuous modes of exercise such as treadmill walking and running and cycle ergometry. A large number of children participate in games activity such as hockey, rugby or football. In a study conducted for Sport England by MORI examining trends in sport participation in young people between 1994 – 2002 (Sport England, 2003) games activity was found to be the most frequently undertaken activity in school PE lessons. In addition football was found to be the most popular extra curricular sport with 15% of young people taking part in 2002. Furthermore games activity may be a mode of exercise that children can sustain for long periods. Therefore it is important to examine what effect this mode of exercise has on postprandial lipaemia. The studies presented in this thesis sought to examine the effect of both continuous and intermittent modes of exercise on blood lipid and lipoprotein metabolism in adolescents and young adults, with particular emphasis on metabolism in the postprandial state. It was hypothesised that those adolescents with high cardiorespiratory fitness levels would have the most favourable blood lipid-lipoprotein profiles and that if the energy expenditure of the continuous and intermittent exercise sessions were high enough, they would both attenuate postprandial lipaemia in adolescents and young adults.

The thesis is presented in 8 main Chapters:

- Chapter 2 presents a review of literature which provides a brief overview of lipid and lipoprotein metabolism, describes the changes in lipid and lipoprotein concentrations during maturation, examines the effect of exercise on lipid and

lipoprotein metabolism in children and adolescents and the effect of exercise on postprandial lipaemia in adults.

- In Chapter 3 general procedures, equipment and methods of analysis used during the experimental studies are described.
- In Chapter 4 the preliminary work undertaken before the main studies is presented. Details are given of a study conducted to evaluate the use of the Reflotron (Boehringer Mannheim, Germany) to determine lipid and lipoprotein concentrations and to examine if there are any difference in concentrations obtained when using venous and capillary blood. Information is also provided of a comparison of oxygen uptake measurements made using douglas bags, carried on the back via a rucksack or connected to a Rack.
- In Chapter 5 the relationship between cardiorespiratory fitness and blood lipid-lipoprotein profile in adolescents is examined.
- Chapter 6 investigated the effect of games activity on postprandial lipaemia in young adults.
- The effect of exercise on postprandial lipaemia in adolescents was assessed in Chapter 7.
- The final Chapter 8 draws together the findings from the experimental work and examines the implications of these findings.

Chapter 2: Review of Literature

2.1 Introduction

The aim of this chapter is to describe the scientific rationale for the studies presented in this thesis. Firstly the reason why it is important to examine the effects of exercise on lipid metabolism is examined. Then there is a brief overview of lipid and lipoprotein metabolism, with particular emphasis on the transport and metabolism of triacylglycerol (TAG) in the circulation. The next section explains how lipid and lipoprotein concentrations change during maturation. The final section reviews the literature concerning the influence of exercise on lipid and lipoprotein metabolism and outlines possible mechanisms for these effects.

2.2 The Relationship between Physical Activity, Cardiovascular Fitness and Health in Children

Before examining the relationship between physical activity, cardiovascular fitness and health it is important that these three terms are defined The World Health Organization (1948) has defined health as "a state of complete physical, mental and social well being and not merely the absence of disease or infirmity". Physical activity is considered to be a behaviour (Dishman, 1990) whereas fitness is an attribute (Montoye *et al.*, 1996; Rowlands *et al.*, 1997). It has been proposed (Caspersen *et al*, 1985) that physical activity is "any bodily movement produced by skeletal muscles that results in energy expenditure", whereas physical fitness is "a set of attributes that people have or achieve that relates to the ability to perform physical activity". It might be useful at this stage to also define exercise (or exercise training). Exercise is defined as a "sub-category of leisure-time physical activity in which planned, structured and repetitive bodily movements are performed to improve or maintain one or more components of physical fitness" (Howley, 2001). The definition of exercise is not all encompassing, as opposed to that of physical activity. It does not include walking/cycling for transport or physical activity performed as a result of work or daily activities (for example walking up stairs, vacuum cleaning, gardening).

Currently, research concerning the relationships between cardiovascular fitness and health in children, compared to that in adults is sparse. In adults, an inverse

relationship has been found between physical activity, cardiovascular fitness and mortality (Paffenbarger *et al.*, 1986; Blair *et al.*, 1989b). In addition, a strong, independent, inverse relationship has been found between high levels of physical activity, cardiovascular fitness and health (Paffenbarger *et al*, 1986; Powell *et al.*, 1987). Studies in adults have also shown that higher cardiovascular fitness or physical activity leads to a reduced risk of CHD (Powell *et al*, 1987; Berlin & Colditz, 1990), stroke (Lee & Blair, 2002), hypertension (Fargard, 2001), type II diabetes (Manson *et al.*, 1992), osteoporotic fractures (Drinkwater, 1994), depression (Biddle & Mutrie, 2001) and some cancers (Friedenreich, 2001). These diseases generally clinically manifest themselves among adults, but not normally in children. Therefore, in order to evaluate the efficacy of physical activity and endurance fitness on the health of children disease risk factors are more typically used. This thesis concentrates on the risk factors for CHD.

Many of the risk factors for CHD are apparent during childhood and adolescence. The principle risk factors (which have been identified in epidemiological studies) include an adverse lipid profile, hypertension, cigarette smoking, diabetes, obesity, sedentary life-style, and familial history of cardiovascular disease (Castelli, 1984) Studies have shown that many children have at least one modifiable risk factor for CHD (Berenson *et al.*, 1980; Boreham *et al.*, 1993). It has also been determined that the extent of both fatty streaks and raised lesions (fibrous plaques and other advanced lesions) in the right coronary artery and in the abdominal aorta was associated positively with non-HDL-cholesterol concentration, hypertension, impaired glucose tolerance, and obesity and associated negatively with HDL-cholesterol concentration (McGill *et al.*, 2000). Furthermore, there is evidence that a child's risk factor status for dyslipidaemia, obesity, hypertension and physical fitness tracks into adulthood (Berenson *et al.*, 1980; Montoye, 1985; Kemper *et al.*, 1990). Tracking has been defined as the "persistence of a behaviour, or attribute over time" and has been said to refer to "the maintenance of a rank order position over time compared to one's peers" (Boreham & Riddoch, 2001). As a result of these observations it is important that the long-range prevention of atherosclerosis by control of the risk factors for adult coronary artery disease should begin in adolescence and young adulthood.

A meta analysis by Hokanson and Austin (Hokanson & Austin, 1996) found that increased plasma TAG concentration is associated with a 32% and 76% increase in

CHD risk in men and women respectively. After adjustment for HDL cholesterol level and other risk factors (such as body mass index and blood pressure), these risks were reduced to 14% in men and 37% in women, but remained statistically significant. It is unclear why increased plasma TAG concentration is associated with a greater risk of CHD in women than in men, although it is important to note that the results were based on more than 46,000 men and only 10,800 women. It is essential because of these findings that ways of reducing hyperlipaemia (including hypertriglyceridemia) are found. In this thesis the effect of physical activity and cardiovascular fitness on blood lipid and lipoprotein metabolism is examined. Although it is instinctively felt that physical activity should be of benefit to children, in contrast to adults there is relatively little research to support this view It has been suggested that there are potentially three main benefits of adequate levels of physical activity/cardiovascular fitness in childhood. The first is a direct improvement of childhood health status and quality of life. The second is a direct improvement of adult health status by delaying the onset of chronic disease in adulthood and finally an increased likelihood of maintaining adequate activity into adulthood, this indirectly enhancing adult health status (Boreham & Riddoch, 2001).

2.3 Lipids

Lipids are long chain hydrocarbons, which are insoluble in water. This is in contrast with carbohydrates, which are largely water-soluble. Lipids have several important functions and are therefore essential for all living cells. They act as fuel molecules, as a concentrated energy store, as structural components of cell membranes and as precursors for steroid hormones. The most plentiful lipid in the body is TAG. It consists of three individual fatty acids, which are each linked by an ester bond to a molecule of glycerol. TAG is the form in which most fat is stored in the human body and in the bodies of other organisms; consequently it is also the major form of fat in food (Frayn, 2003).

2.4 Lipoproteins

Lipoproteins are particles with a highly hydrophobic lipid core and a relatively hydrophilic outer surface. As lipids are hydrophobic compounds, lipoproteins are needed to transport these compounds through the aqueous medium of blood plasma to

the cells were they are needed. A lipoprotein particle consists typically of a core of TAG and cholesteryl ester, with an outer surface layer of phospholipid and free cholesterol. Each particle also has associated with it one or more protein molecules, the apolipoproteins. The apolipoproteins contribute to the stability of the lipoproteins; providing recognition sites for cell membrane receptors and acting as cofactors for enzymes involved in lipoprotein metabolism (Durstine & Haskell, 1994). Apolipoproteins have hydrophobic domains, which "dip into" the core and anchor the protein to the particle, and hydrophilic domains that are exposed at the surface (Frayn, 2003).

Lipoproteins are a heterogeneous group of particles, with different lipid and protein compositions, and different sizes. They are separated into fractions (or groups) based on either electrophoretic mobility, flotation in an ultracentrifuge or on hydrated density. The main fractions are chylomicrons, very low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL). Chylomicron and VLDL particles are rich in TAG and as a result are often referred to as the TAG-rich lipoproteins. They are responsible for the delivery of TAG to tissues from the intestine (chylomicrons) or from the liver (VLDL) to tissues The smaller LDL and HDL particles are involved with the transport of cholesterol to and from cells (Frayn, 2003).

2.5 An Overview of Lipoprotein Metabolism

A large variety of body tissues make demands on the plasma lipid pool e.g. cholesterol is used as a precursor to hormones and bile acids, while TAG is an important energy source for cardiac and skeletal muscle. Lipid metabolism is designed to deal more with the frugality of past times than the relative abundance of today and as a result lipoprotein transport regulatory mechanisms operate most effectively at low plasma lipid concentrations. Most cells have only a limited capacity to store cholesterol or TAG and take steps to limit ingress when they have sufficient stores. As the body does not possess feedback mechanisms to inhibit absorption, intake in excess of requirements leads to the accumulation of lipid in the circulation. This has pathological consequences in individuals at risk, with cholesterol in particular being deposited in blood-vessel wall (Packard & Shephard, 1999).

2.5.1 Pathways of lipid transportation

The transport of lipids through the circulation of the body can be broadly classified into two pathways in which the liver plays a central role as a "clearing house" for either exogenously or endogenously derived lipids (Herz, 1999).

2 5.2 The Exogenous Pathway

The exogenous pathway transports dietary fat. In the postprandial phase TAG and cholesterol that have been ingested are absorbed and re-esterified in the cells of the intestinal wall, and secreted by enterocytes as chylomicron particles, into the intestinal lymph. They then pass through the thoracic duct and enter the general circulation through the left subclavian vein. These secreted chylomicrons, contain a core of TAG and cholesteryl esters covered by a surface layer of phospholipids, cholesterol and apolipoprotein B48 and A1 [for review see Green & Glickman (1981)].

Following their entry into the systemic circulation, chylomicrons acquire apolipoproteins CI CII, CIII and E (Patsch, 1987). These surface components are transferred to chylomicrons from HDL along with free and esterified cholesterol and phospholipids. Apolipoproteins CII and CIII are involved in the postprandial processing of both chylomicrons and VLDL. The major role of apolipoprotein E seems to be the targeting of the chylomicron remnant (the product of chylomicron TAG hydrolysis) for the receptor, serving as a ligand for remnant removal by the liver.

Once the chylomicron has acquired apolipoprotein CII it is capable of activating lipoprotein lipase (LPL - found at the endothelial surface of capillaries in sites such as muscle and adipose tissue), the enzyme responsible for the hydrolysis of TAG in chylomicron and VLDL particles. Hydrolysis of chylomicrons results in smaller, relatively protein enriched particles with a redundant surface coating of free cholesterol and phospholipid. As the TAG component becomes progressively smaller, surface materials (phospholipids, cholesterol and apolipoprotein CII and CIII) are transferred to HDL to maintain the stability of the chylomicron particle. The transfer of apolipoprotein CII, together with increasing inaccessibility of core TAG for the lipoprotein lipase active site, results in cessation of further TAG removal (Sethi *et al.*, 1993).

The chylomicron particles are now known as chylomicron remnants. They are relatively enriched in cholesteryl ester, since they have lost their TAG, and are potentially harmful. They become ligands for a receptor in the liver. The nature of this receptor is uncertain but it may be the α2-macroglobulin receptor. Thus dietary TAG is delivered to the tissues, some unesterified cholesterol enters the HDL fraction, and some cholesteryl ester is delivered, in the remnant particles, to the liver (Frayn, 2003). A small number of large chylomicron remnant particles are also removed from the circulation by peripheral tissues implying that some removal of TAG-rich lipoproteins can occur via whole-lipoprotein particle removal as well as delipidation via the LPL pathway (Karpe, 1997).

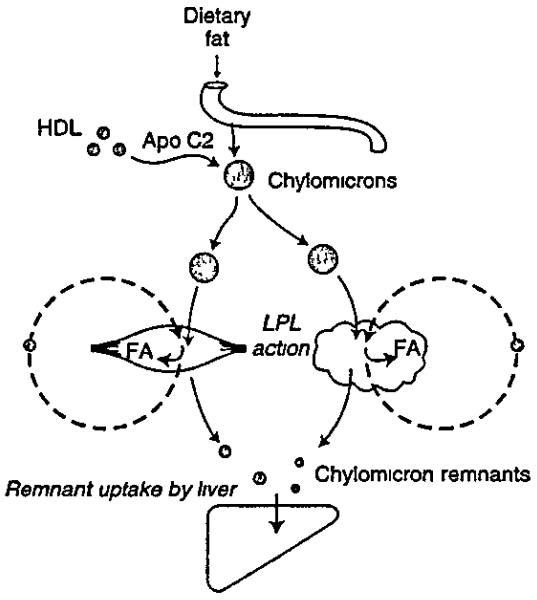

Figure 2.1 The Exogenous Pathway. Reproduced from Frayn, (1996)

2.5.3 The Endogenous Pathway

The endogenous pathway (also known as VLDL metabolism) distributes TAG from the liver to other tissues. VLDL particles are synthesised and then secreted by the liver, and contain TAG, cholesteryl ester, apolipoprotein B100 and small amount of apolipoproteins E and C. They have a surface coat, like all lipoprotein particles, of phospholipids and unesterified cholesterol. The content of apolipoprotein E and C rapidly increases in the plasma by transfer from other lipoproteins, mainly HDL.

These large VLDL particles transport hepatically derived TAG from the liver to peripheral target tissues where they, as with the intestinally derived TAG in chylomicrons, become a substrate for LPL in capillary beds. The VLDL particles shrink as a result of the hydrolysis of its TAG core, loses apolipoprotein C and acquires apolipoprotein E. The VLDL remnants that result have two possible fates. They can be removed from the circulation by binding to hepatic lipoprotein receptors (this interaction is mediated by apolipoprotein E) or undergo further hydrolysis and give rise to intermediate density lipoproteins (IDL) and finally to LDL (Herz, 1999). The lipolysis of VLDL particles is much slower than that of chylomicrons (Havel, 1997) and their half-life in the circulation is two to four hours in normolipidaemic individuals (Durstine & Haskell, 1994).

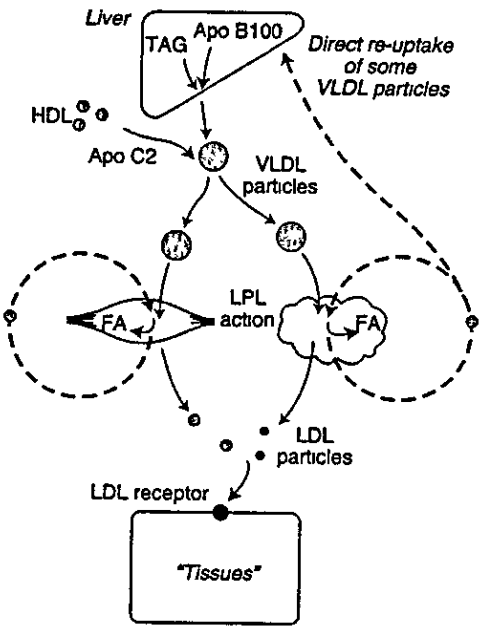

Figure 2.2 The Endogenous Pathway. Reproduced from Frayn, (1996)

2.5.4 LDL Metabolism and Regulation of Cellular Cholesterol Content

LDL particles have a relatively long half-life in the circulation of about 2.5 days (Durstine & Haskell, 1994). During this time they are metabolically relatively stable. They leave the circulation mainly through uptake into various tissues by the LDL receptor and deliver cholesterol to tissues. This increases the cellular content of cholesterol in tissues that take up LDL particles. This has the effect of suppressing the biosynthesis of cholesterol (which can occur in all nucleated cells) primarily by

suppression of the enzyme hydroxymethylglutaryl-CoA reductase (HMG-CoA reductase). Secondly, synthesis of new LDL receptors is suppressed, and the number of receptors expressed on the cell surface is reduced. Therefore, the increase in cellular cholesterol content caused by uptake of LDL-cholesterol by the LDL receptor is self-limiting (Frayn, 1996).

However some cells, particularly macrophages, express different receptors which take up LDL particles e g. the scavenger receptor. This can cause the macrophages of people with a high plasma LDL-cholesterol concentration to become excessively cholesterol laden. This can be the beginning of the process of atherosclerosis (Patsch, 1987).

2.5.5 HDL and Reverse Cholesterol Transport

HDL is the smallest and most dense of the lipoproteins and transports cholesterol away from peripheral tissues back to the liver for ultimate excretion. This is termed "reverse cholesterol transport". Unlike all other plasma lipoproteins HDL is not made as a spherical, mature lipoprotein. HDL particles are secreted by the liver as discoidal structures. These discoidal particles consist mainly of phospholipid and apolipoprotein A1. The metabolism of HDL is linked with the metabolism of TAG-rich lipoproteins as the discoidal HDL particles receive unesterified cholesterol, which is released as " excess surface material" during the action of LPL on the TAG-rich lipoproteins. They also obtain unesterified cholesterol by interaction with cells, possibly via a specific receptor, to form mature spherical HDL particles. Further transfer of unesterified cholesterol from TAG-rich lipoproteins produce particles known as HDL_2 particles. The liver can take up the cholesteryl ester in these particles by a number of mechanisms. There may be receptor-mediated uptake of large HDL particles, which contain apolipoprotein E. In addition, there seems to be hepatic uptake of cholesteryl ester from the larger HDL particles, perhaps involving hydrolysis of cholesteryl ester by hepatic lipase. Some of the cholesteryl ester from larger HDL particles is also transferred to the TAG-rich lipoproteins. The smaller HDL particles that result are known as HDL_3 and are ready to accept further cholesterol from peripheral tissues. Thus cholesterol is transferred from peripheral tissues to the liver, from where it can be excreted as cholesterol and as bile salts (Frayn, 2003).

An individual's ability to metabolise TAG rich lipoproteins is a strong determinant of his or her plasma HDL cholesterol and, in particular, HDL_2 cholesterol concentration. This could mean that the negative association between HDL cholesterol and risk of developing CHD might represent the relationship between HDL and the metabolism of TAG-rich lipoproteins (a potentially atherogenic process) rather than an anti-atherogenic effect of the particles themselves (Miesenböck & Patsch, 1992).

2.5 6 Integration of TAG-rich Lipoprotein Metabolism

In the fasted state endogenous VLDL are the only TAG-rich lipoprotein in the circulation. After consumption of a meal containing fat, chylomicrons enter the circulation and mix with VLDL in the circulation and these particles then compete for clearance by the same lipolytic pathway (Brunzell *et al.*, 1973). The presence of the chylomicrons impedes the metabolism of VLDL and causes TAG-rich lipoproteins of endogenous origin to accumulate in plasma in the postprandial state. It has been proposed that this increase in concentration of VLDL during postprandial lipaemia results from the greater affinity of the larger chylomicrons for LPL on the surface of capillary endothelial cells, thereby increasing VLDL residence time in the blood. Thus, the residence time of chylomicron TAG in the blood is normally about 5 min, whereas that of VLDL-TAG is several-fold longer, even at low VLDL concentrations. As VLDL-particle concentrations increase, however, these particles can compete to a greater extent for LPL. This indicates that the postprandial accumulation of VLDL particles is not due to increased VLDL secretion from the liver but rather due to the competition with chylomicrons for hydrolysis of TAG by LPL (Havel, 1997).

2 5 7 Regulation of Postprandial TAG Metabolism

A major determinant of postprandial lipaemia under the majority of circumstances is the activity of LPL, which is considered to be the rate-limiting enzyme in TAG hydrolysis (Taskinen & Kuusi, 1987). LPL is subject to reciprocal regulation in adipose tissue and skeletal muscle with activity increasing in the former tissue, but decreasing in the latter, after ingestion of a meal (Lithell *et al*, 1978). Thus, the relative contribution of (unexercised) skeletal muscle to lipoprotein-TAG clearance is greater in the fasted state, than the postprandial state. After ingestion of a meal containing carbohydrate or protein insulin concentrations increase and facilitates LPL

activity in adipose tissue while suppressing LPL activity in skeletal muscle (Kiens *et al.*, 1989). Therefore, it is likely that insulin plays a major role in the regulation of LPL in the fasted and postprandial states.

Insulin is an important coordinator of lipoprotein metabolism. As well as regulating LPL activity it suppresses hormone sensitive lipase (HSL) activity and is a potent stimulator of re-esterification of fatty acids in adipose tissue (Coppack *et al.*, 1992). HSL action remains suppressed for a number of hours after a meal. This leads to suppression of NEFA release from adipose tissue in the postprandial state, which leads to low plasma NEFA concentrations early in the postprandial state. This acts to reduce hepatic VLDL secretion by reducing the fatty acid flux to the liver (Frayn *et al.*, 1997).

After hydrolysis by LPL, fatty acids derived from the TAG core of chylomicron and VLDL particles are taken up and re-esterified in the peripheral tissues. Entrapment of these re-esterified fatty acids is high in skeletal muscle but in adipose tissue a proportion of these fatty acids are not esterified and "spill-over" into the plasma NEFA pool (Coppack *et al*, 1990; Frayn *et al.*, 1994). This appears to be a highly regulated process. After an overnight fast there is almost no retention of LPL derived fatty acids in adipose tissue and fatty acid entrapment rises to 70-90% within 90 minutes of ingestion of a mixed-meal containing moderate or large amounts of fat (when insulin and glucose concentrations are high) (Frayn *et al.*, 1995). Four to five hours after meal ingestion (when TAG extraction is maximal) less than 50% of LPL-derived fatty acids are retained in adipose tissue, with the remainder spilling over into the circulation. Thus, plasma NEFA concentrations rise during the mid-to-late postprandial period, after the initial suppression, predominately due to the influx of LPL-derived NEFA. This increases the fatty acid flux to the liver and may stimulate VLDL production in the postprandial state (Frayn, 1996).

2.6 Blood Lipids and Lipoproteins During Childhood and Adolescence

Blood cholesterol, LDL, and TAG concentrations are very low at birth and then they increase rapidly during the first year of life (Kunze, 1983). During childhood and adolescence lipid and lipoprotein concentrations generally remain relatively stable with few differences in concentration between males and females (Åkerblom *et al.*,

1984, Armstrong *et al.*, 1992). The exception is HDL, which declines in males during adolescence and is significantly lower in males than in females (Armstrong *et al.*, 1992). During the pre-school years, year-to-year correlations for plasma cholesterol, TAG, and blood pressure are generally low. However tracking improves with age during the school years, and year-to-year correlations increase after puberty. As plasma lipids increase with age it seems that tracking is better during and after puberty than during childhood. Acceptable tracking of total cholesterol and LDL cholesterol has been observed, but lower tracking of TAG and HDL cholesterol. However extreme plasma lipid values track better and very high lipid values tend to remain high throughout childhood (Webber *et al* , 1991).

2.7 Physical Activity, Cardiorespiratory Fitness and Blood Lipid-Lipoprotein Profile in Children

The literature concerning the response of young people's lipid and lipoprotein concentrations to physical activity and cardiorespiratory fitness is relatively scarce when compared with the number of adult studies. A recent review (Eisenmann, 2002) which examined the blood lipid and lipoprotein concentrations in child and adolescent athletes concluded that when young athletes were compared with controls, they had similar levels of total cholesterol, lower levels of TAG and LDL cholesterol and higher levels of HDL cholesterol. This is consistent with the conclusions of other reviewers who have considered the differences between "trained" and "untrained" young people (Armstrong & Simons-Morton, 1994; Tolfrey *et al.*, 2000). The mean difference between young athletes and controls for total cholesterol was found to be 0.26 $mmol.l^{-1}$, for LDL cholesterol and TAG mean differences ranged from 0.13 to 0.65 $mmol.l^{-1}$ and 0.23 and 0.39 $mmol.l^{-1}$ respectively. Mean differences for HDL cholesterol ranged from 0.01 to 0.44 $mmol.l^{-1}$ (Eisenmann, 2002) In some studies were differences in individual lipids and lipoproteins have not been significant between groups, differences emerge when combined to form atherogenic ratios (for example Thorland and Gilliam (Thorland & Gilliam, 1981)).

These cross-sectional data needs to be interpreted with caution as few studies defined their criteria of "trained", "control/normal", or "inactive" participants and little indication was given of selection procedures. This is compounded by small sample size and information is rarely given on participants training intensity, frequency,

duration of sessions, or length of time they have been training. In some studies male and female data have not been analysed separately (Smith *et al*, 1986). This can cause problems when considering potential maturation effects during adolescence. Pre-training lipid and lipoprotein profiles are also generally not reported. Furthermore, it is often impossible to ascertain whether changes in blood lipids and lipoproteins have been accompanied or influenced by changes in cardiopulmonary fitness, or changes in body composition or diet (Armstrong & Simons-Morton, 1994; Tolfrey *et al.*, 2000).

It is important to note that in adults cardiovascular fitness, which is commonly measured by determining peak $\dot{V}O_2$ is significantly associated with physical activity (Blair, 1994). However, in children the degree of associate has been found to be moderate with correlations ranging from 0.3 to 0.8 (Pate *et al.*, 1990). It has been concluded that the majority of the available evidence suggests that peak $\dot{V}O_2$ is not related to total cholesterol, HDL cholesterol, LDL cholesterol or TAG in children and adolescents (Tolfrey *et al.*, 2000). However there are a few exceptions to this general finding (Verschuur *et al.*, 1984; Atomi *et al.*, 1986; Smith *et al*, 1986). When associations are found between lipids-lipoprotein and physiological variables, the strength of these associations may depend upon the sex of participants (Gilliam *et al.*, 1981; Tolfrey *et al.*, 1999). In a recent study using multiple linear regression Tolfrey and colleagues (Tolfrey *et al.*, 1999) reported that peak $\dot{V}O_2$ was related to TAG, HDL cholesterol, LDL cholesterol/HDL cholesterol, and total choleterol/HDL cholesterol in prepubertal girls. None of these associations were demonstrated in the maturity-matched boys. It was suggested that differences in participant characteristics, lipid-lipoprotein concentrations and dietary composition could explain the apparent sex effect (Tolfrey *et al.*, 1999).

It has been noted (Tolfrey *et al.*, 2000) that both body composition and methods employed to assess cardiorespiratory fitness are important factors that need to be considered when interpreting results from correlational studies as the relationship between percentage body fat (or body mass) and the lipid-lipoprotein profile in children and adolescents is well established (Rowland, 1996; Armstrong & Welsman, 1997). It has been recommended in adults that if favourable changes in blood lipid-lipoprotein profile are sought a reduction in body mass is necessary (Tran *et al.*,

1983). It has not been conclusively determined it this is the case in children and adolescents.

The effects of habitual physical activity on blood lipid-lipoprotein have been reviewed (Armstrong & Simons-Morton, 1994) and in general no significant differences have been found, although there are perhaps some indicators that active children may have higher HDL cholesterol and lower TAG concentrations. The results may have been confounded by the difficulty in estimating adolescents physical activity levels and possibly by interactions between physical activity, body fatness and blood lipid-lipoprotein profile (Armstrong & Simons-Morton, 1994). It is therefore, important to attempt to control for these variables. One way of doing this is to use partial correlations (Tolfrey *et al.*, 2000).

There are currently relative few training studies examining the effect of exercise training on blood lipid–lipoprotein profile in children and/or adolescents and those that have been undertaken have yielded mixed results. In one study, 50 boys aged 11-17 years, were randomly assigned to a physical conditioning programme or a control group (Linder *et al.*, 1983). The exercise group participated in an 8-week aerobic exercise program that included four 30 min sessions per week. No changes in plasma lipid and lipoprotein concentrations were observed at the end of the exercise programme. In another study it was reported that a 10-week, moderate and high intensity exercise training programme had no effect on the plasma lipoprotein and lipid concentrations in late adolescents (Hunt & White, 1980). However although Savage and colleagues (Savage *et al.*, 1986) did not find evidence for an exercise-training effect in two groups of 8 – 9 year old boys exposed to 10 weeks of low (40% peak $\dot{V}O_2$) or high (75% peak VO_2) intensity training (walking, jogging, and running), significant decreases in HDL cholesterol concentrations occurred in both groups after training. Another study performed by Fisher and Brown (Fisher *et al.*, 1982) observed a significant decrease in serum cholesterol concentrations and an increase in HDL cholesterol concentrations in 7th grade children after 30 min of physical activity, 5 days per week over 12 weeks.

Examination of the available data reveals that pre-training concentrations of lipids and lipoproteins may be strong predictors of percentage change when assessing the effects of exercise intervention. This has been called the "ceiling" or "floor" effect (Tolfrey

et al, 2000); that is studies reporting an increase in HDL cholesterol had participants with baseline concentrations that were significantly lower than those in "unsuccessful" programmes.

These data suggest that exercise training may be an effective means of normalising unfavourable plasma lipid-lipoprotein concentrations and is consistent with the meta-analysis in adults by (Tran *et al.*, 1983), which indicated that younger participants with higher peak $\dot{V}O_2$ and less-abnormal lipid concentrations can expect to experience limited changes in lipids and lipoproteins with training. To date the majority of studies assessing lipid changes with training have principally involved fit, healthy, motivated children. In children who are obese or sedentary increases in physical activity may have a greater beneficial effect on blood lipid profiles (Rowland, 1990).

The evidence from the majority of these studies does not suggest any changes in the lipid-lipoprotein profile of children and adolescents following exercise training. It is possible that the primary reason for a lack of change in the lipoprotein profile of the children seen in some studies has been due to inadequate exercise volume or dose (Tolfrey *et al.*, 2000). In a review, Superko (1991) suggested that the overall energy expenditure might determine whether it is possible to favourably alter the lipid-lipoprotein profile in adults. This view has been supported by training studies in adults (Hardman *et al.*, 1989; Hardman & Hudson, 1994). Whether this is true of children has yet to be thoroughly evaluated. Many training programmes have been completed in school physical education classes in order to increase ecological validity (Deveaux *et al.*, 1986; Rowland *et al.*, 1996). As a result school class length is generally reported rather than actual exercise duration and information regarding exercise intensity is usually not provided. Exercise volume or dose is therefore difficult to determine. In a training study performed with prepubertal children where participants were required to perform 3 x 30 min sessions of cycling a week for 12 weeks at approximately 80% of peak heart rate significant alterations in the lipid-lipoprotein profile were reported (Tolfrey *et al*, 1998). Heart rate was continuously monitored throughout each exercise training session and it was found that unless the exercise intensity was continuously monitored it tended to fluctuate considerably, and that the feedback provided by the monitors served to encourage and motivate the children. It was suggested that unmonitored children would most likely train in an

interval fashion. A study has recently been performed to examine the effect of exercise volume on pre and early pubertal children's lipid-lipoprotein profile (Tolfrey *et al.*, 2004). Thirty-four children (15 girls) completed 12 weeks of exercise training, preceded by a 12 week control period. Sixteen (7 girls and 9 boys) expended an additional 422 ± 5 $kJ.kg^{-1}$ body mass (100 $kcal.kg^{-1}$ per session), whereas 18 (8 girls and 10 boys) expended an additional 586 ± 7 $kJ.kg^{-1}$ body mass (140 $kcal.kg^{-1}$ per session) as a result of the training program. They all exercised on three nonconsecutive days per week at 80 ± 1% peak heart rate. Exercise duration was individualised to match energy expenditure targets. Additional energy expenditure of 422 or 586 $kJ.kg^{-1}$, as a direct result of aerobic exercise training over a 12 week period, did not cause significant alterations in the lipid-lipoprotein profile in pre and early pubertal children. The authors suggested that this might indicate that the exercise volume was insufficient, the lipoprotein profiles of the majority of children in this study were classified as "desirable," or more likely a combination of these factors. In a recent study in young distance runners (27 male, 17 female aged 9 – 18 years) it was found that despite their relatively large training volumes (recorded longitudinally) the development of blood lipids was similar to youth in the general population (Eisenmann *et al*, 2001a). In contrast to observations in adult endurance athletes, young distance runners did not possess a superior blood lipid profile except for HDL cholesterol in the younger age group. Furthermore, from a cross sectional study the same research team found that training volume was not a significant predictor of the lipid-lipoprotein profile in a group of 70 endurance trained athletes (48 male, 22 female aged 10 – 19 years) (Eisenmann *et al.*, 2001b). The literature on the effects of exercise training on children is thus equivocal. Further research is clearly warranted.

2.8 The Importance of Examining Lipid and Lipoprotein Metabolism in The Postprandial State

People typically consume multiple meals during the day with the result that additional food is ingested long before the metabolic disturbances associated with previous meals have subsided. Consequently people can spend perhaps two thirds of the day in the postprandial state. It has therefore been said that these repeated metabolic challenges represent the "normal" physiological state (Gill & Hardman, 2003). Postprandial lipaemia is associated with the metabolic syndrome (Jeppesen *et al.*, 1995), which is a clustering of symptoms including visceral adiposity, hyperlipaemia,

insulin resistance, glucose intolerance and hypertension. All of these symptoms increase the risk of CHD. It has been proposed that atherosclerosis is a postprandial phenomenon with the remnants of postprandial lipoproteins directly infiltrating the arterial wall leading to the accumulation of atheromatous plaques (Zilversmit, 1979). In addition, elevated postprandial TAG concentrations are believed to affect endothelial function (Vogel *et al*, 1997). Hence interventions that reduce postprandial lipaemia may play a role in delaying the development of atherosclerosis.

Recently studies have been published examining postprandial lipaemia in children and young adults. In one study a relationship was found between delayed postprandial fat clearance and a family history of early-onset ischemic heart disease in young adults (mean age 24.8 years) (Uiterwaal *et al*, 1994). However in contrast to this finding another study found no significant differences in areas under the postprandial curves for TAG, chlyomicron and chylomicron remnant retinyl palmitate between participants (aged 15 – 30 years) who had parents with CHD on or before the age of 60 years (Slyper *et al.*, 1998) and participants who did not. When the factors which could be used to predict postprandial TAG response in children were considered it was found that a delayed postprandial TAG response to a fat load was associated with the combination of high fasting TAG and low HDL cholesterol concentrations and therefore that the predictors of postprandial TAG concentrations may be similar in children and adults (Couch *et al.*, 2000). The effects of body composition and fat distribution on postprandial lipaemia in both obese and non-obese adolescents has also been examined (Moreno *et al.*, 2001). A significant increase in serum TAG concentrations was observed 2 to 4 h after an oral fat load in both obese and non-obese adolescents. When postprandial lipaemia was compared in adolescents having a central pattern of fat distribution (high levels of fat in the waist and upper abdominal area, assessed using hip to waist circumference ratio) with those having a peripheral pattern of fat distribution (high levels of fat in the arms and legs, assessed using triceps to subscapular skinfold ratio) higher variables related to postprandial lipaemia were observed in those having a central pattern of fat compared with those with a peripheral pattern. It was concluded that the pattern of distribution of adipose tissue may be more important for lipid metabolism disturbances than total adipose tissue *per se* in adolescents. Postprandial hyperlipidaemia in response to a fat loading test is present in adolescents with type II diabetes who already have fasting hypertriglyceridemia (Umpaichitra *et al*, 2004). In addition the degree of insulin

resistance as an underlying abnormality (not diabetes *per se*) determines the degree of postprandial lipemia.

The most consistent alteration of exercise/physical activity on lipid-lipoprotein profile is an increased plasma concentration of HDL cholesterol in both adults and children (Durstine & Haskell, 1994; Armstrong & Welsman, 1997; Leon & Sanchez, 2001). The efficient metabolism of TAG rich lipoproteins is a major determinant of HDL concentration (Miesenböck & Patsch, 1992). The efficiency of TAG metabolism is particularly evident during the postprandial period and demonstrates how HDL cholesterol concentration can be viewed as a marker of TAG metabolism. As mentioned in Section 2.5.6 after the ingestion of a meal intestinally derived chylomicrons enter the circulation and compete with VLDL for clearance by the same lipolytic pathway. This results in high concentrations of TAG rich lipoproteins (chylomicrons and VLDL) in the blood stream. This leads to accelerated transfer of esterified cholesterol from HDL and LDL to TAG rich lipoproteins, with TAG transferring in the opposite direction. This reduces the HDL cholesterol concentrations in the blood and leads to TAG enriched, cholesteryl ester-depleted HDL and LDL particles. The TAG content of these particles is then hydrolyzed by hepatic lipase, resulting in small HDL particles which are removed from the circulation at an enhanced rate (Rashid *et al.*, 2002) and small dense LDL particles which are atherogenic (Griffin, 1997) (the accumulation of small dense LDL and low HDL cholesterol concentrations is known as "atherogenic lipoprotein phenotype"). Thus, examining TAG metabolism in the postprandial state is potentially an ideal way to gain information regarding the mechanisms by which exercise influences blood lipid-lipoprotein profile.

2.9 Exercise and postprandial lipaemia

The effect of exercise on postprandial lipaemia has yet to be investigated in children However, cross-sectional studies in adults have shown that regular exercisers have lower postprandial lipaemia (Cohen *et al.*, 1989) and/or increased TAG clearance (Sady *et al.*, 1986; Cohen *et al*, 1989; Podl *et al.*, 1994) compared with sedentary controls. The data from these studies have recently been reviewed and overall, the evidence from cross-sectional studies is that postprandial lipaemia following an oral fat challenge is 27 – 59% lower, and removal rates of intravenous TAG 26 – 92%

higher, in endurance-trained individuals than in untrained or sedentary controls (Hardman & Herd, 1998). Trained people exhibit these characteristics despite their low levels of body fat, the tissue with highest LPL activity per unit mass, suggesting that the quantity and/or the quality of their skeletal muscle is important (Hardman & Herd, 1998). Postprandial lipaemia has also been investigated in the presence and absence of effects of a recent moderate intensity exercise session in endurance-trained and untrained middle-aged women (Tsetsonis *et al.*, 1997). The exercise session was performed approximately 18 h before ingestion of the test meal. It was found that postprandial lipaemia decreased by 16% in the untrained group and 30% in the trained group when the exercise session was performed. The greater decrease seen in the trained group may imply that the chronic and acute effects of exercise interact synergistically. It was also noted that after the participants had refrained from exercise for almost three days before the ingestion of the test meal, there was no difference in postprandial lipaemia between trained and untrained participants.

Similar reductions in postprandial lipaemia have also been reported by some training intervention studies (Thompson *et al.*, 1988; Weintraub *et al.*, 1989). However Aldred and colleagues (Aldred *et al*, 1995) observed no changes in postprandial lipaemia in middle-aged female participants after a 12 week brisk walking programme despite clear increases in endurance fitness and an indication that insulin sensitivity was enhanced. It is interesting to note that the post-training oral fat tolerance test in this study was performed after two days of inactivity to eliminate the confounding influence of acute exercise on the plasma TAG response. These findings support the hypothesis that in the absence of a recent exercise session, exercise training does not improve TAG metabolic capacity. However the intensity of training (60% peak $\dot{V}O_2$) might not have been sufficient to enhance TAG metabolic capacity.

Detraining studies can also reveal much about the nature of exercise training induced changes to lipoprotein metabolism. A study performed by Hardman and colleagues (1998) included ten athletes, (nine male, seven distance runners, two triathletes and one cyclist) five of whom competed at international or regional level, who where asked to refrain from training for a week. Oral fat tolerance tests were performed 15 h, 60 h and 6.5 days after the athletes' last exercise session. Postprandial lipaemia was 35% higher 60 h after the cessation of exercise and 42% higher after 6.5 days of detraining when compared with values measured 15 h after exercise. This data shows

the important contribution of a recent session of exercise to the low levels of postprandial lipaemia that are characteristically seen in endurance-trained people.

A single exercise session performed some hours prior to ingestion of a test meal has also been shown to attenuate postprandial lipaemia (Aldred *et al.*, 1994). It was found that two hours of brisk walking at 30% of peak VO_2 on the afternoon reduced the lipaemic response to a test meal consumed the following morning by 31%, in a group of young normolipidaemic men and women. It has also recently been demonstrated that exercise sessions of the same energy expenditure, but different intensity, elicit similar attenuation in postprandial lipaemia (Tsetsonis & Hardman, 1996a). In addition, three hours of exercise at 30% peak $\dot{V}O_2$ of induced a similar reduction as 90 min at 60% of peak $\dot{V}O_2$ (Tsetsonis & Hardman, 1996b). These two studies seem to suggest that it is the energy expenditure of an exercise session that determines the magnitude of the exercise induced reduction in postprandial lipaemia, not the exercise intensity.

The most recent physical activity guidelines for adults have promoted the accumulation of multiple bouts of aerobic activity (minimum duration of 10 min accumulated throughout the day) as well as continuous aerobic activity as a means of achieving the recommendation 30 min of moderate intensity activity a day on most, preferably all days of the week (Pate *et al.*, 1995; ACSM, 1998; Department of Health, 2004). Although this concept of accumulation has been widely publicised, there is still limited scientific evidence to support this recommendation. Four studies have examined the effects of accumulated/intermittent activity on postprandial lipaemia. The most recent of these studies examined the effect of accumulating 6 min bouts of exercise until participants had accumulated a gross energy expenditure of 4.2 MJ and found that postprandial lipaemia was reduced by 18% compared with rest (Miyashita *et al.*, Unpublished). In another study, three 10 min bouts of exercise (20 min recovery between bouts) were found to be more effective than one 30 min bout (Altena *et al.*, 2004). It was suggested by the authors that the three 10 min bouts of exercise could have elicited a slightly greater energy expenditure compared with the single 30 min bout of exercise as a result of excess postexercise oxygen consumption. A similar study found that three 10 min bouts accumulated throughout one day were as effective as one 30 min bout (Murphy *et al.*, 2000). Lastly three 30 min bouts of

exercise accumulated over the course of a day were found to be as effective as one 90 min bout (Gill *et al.*, 1998).

Recently two groups (Petitt *et al.*, 2003; Burns *et al.*, In Press) have examined the effect of resistance exercise on postprandial lipaemia and they have found conflicting results. A 14% reduction in postprandial lipaemia was seen following a bout of resistance exercise involving three sets of 10 repetitions of 10 exercises performed at 10 repetition maximum (Petitt *et al.*, 2003). In contrast, when participants completed a greater volume of exercise (four sets of 10 repetitions of 11 exercises) but at a lower intensity (80% of 10 repetition maximum), no reduction was seen (Burns *et al.*, In Press). It is clear that the effect of resistance exercise on postprandial lipaemia warrants further investigation.

Taken collectively the studies outlined above suggest that exercise can markedly reduce postprandial lipaemia even in untrained participants. It is important to determine if exercise can reduce postprandial lipaemia in children and adolescents

2.10 Potential Mechanisms Responsible for the Exercise Induced Reduction in Postprandial Lipaema

Although the mediating factors responsible for the exercise induced reduction in postprandial lipaemia are not completely understood, it seems likely that two complementary mechanisms are involved. One is thought to involve increased TAG clearance from the blood stream. The other is thought to be a reduced rate of appearance of TAG rich lipoproteins (either chylomicrons and/or VLDL)

Highly trained individuals have been shown to exhibit enhanced rates of TAG clearance when compared with untrained controls (Sady *et al*, 1988; Podl *et al.*, 1994). This enhance rate of TAG clearance is likely to be mediated by an up regulation of LPL activity. Post heparin plasma LPL activity (that is LPL activity measured in blood plasma collected 10 min after injection of heparin sodium) has been reported to be higher in endurance trained individuals than in sedentary controls (Podl *et al.*, 1994). This reflects increased activity in skeletal muscle and possibly in adipose tissue (Nikkilä *et al.*, 1978). Higher plasma LPL activity has also been seen as a result of exercise training (Thompson *et al.*, 1988; Weintraub *et al.*, 1989). This

increase could be due to increased capillaristion in muscle (Kiens *et al.*, 1989). A positive relationship has been found between plasma LPL activity and clearance rate in an intravenous fat tolerance test in male (Sady *et al*, 1988) and female runners (Podl *et al*, 1994) (Spearmans' rank order correlation coefficients 0.74 and 0.61 respectively). Thus it is likely that increased LPL mediated clearance of TAG from TAG rich lipoproteins could be an important contributor to the low levels of postprandial lipaemia seen in endurance trained people and to the attenuation in postprandial lipaemia seen following exercise.

There is however information to refute this theory. A recent study found that 90 min of brisk walking significantly reduced postprandial lipaemia but the same amount of exercise did not increase clearance of an intravenous lipid emulsion (Gill *et al.*, 2001a). In addition, another study demonstrated that 90 min of moderate exercise did not increase mean skeletal muscle LPL activity 18 h following exercise despite significantly reducing postprandial lipaemia (Herd & Hardman, 1997). Moreover a study using arterio-venous difference methods demonstrated that two hours of moderate exercise did not significantly increase absolute TAG uptake across the leg (which is mainly skeletal muscle) 18 h post exercise, despite again significantly attenuating postprandial TAG concentrations (Malkova *et al.*, 2000). These studies suggest that mechanisms other than increased TAG uptake into skeletal muscle are likely to contribute to the TAG reduction seen after moderate intensity exercise. Prior exercise has not been shown to delay the time to peak postprandial chylomicron TAG concentration (Malkova *et al.*, 2000; Gill *et al.*, 2001b) which indicates that the rate of chylomicron appearance is not reduced. In addition, gastric emptying has not been found to be reduced on the day following exercise (Gill *et al.*, 2001a; Gill *et al.*, 2001b). Therefore, it seems unlikely that a reduced rate of chylomicron appearance is a contributor to the postprandial TAG reduction seen as a result of exercise. Therefore it is logical to assume that reduced hepatic VLDL secretion could make a contribution. Although there is currently no direct evidence to support this theory in humans, studies in rats have shown that hepatic VLDL production rates were reduced by exercise training (Fukuda *et al.*, 1991). Lipid production rates and the production rates of other metabolic markers were measured in this study using liver perfusion methods. In this study increased hepatic ketone body production was also reported. This suggests increased hepatic fatty acid oxidation. It was concluded from these results that exercise altered the partitioning of fatty acids in the liver between

oxidation and esterification pathways and this resulted in the lower production of VLDL. Since increased 3-hydroxybutyrate concentrations (a marker for increased hepatic fatty acid oxidation) have been reported in conjunction with reduced postprandial TAG concentrations in response to exercise in humans (Malkova *et al*, 2000; Gill *et al.*, 2001b) it is possible that reduced hepatic VLDL secretion could play a role in the attenuation in postprandial lipaemia seen as a result of exercise. The relative contributions of enhance TAG clearance and reduced VLDL secretion to the attenuation of postprandial lipaemia has yet to be fully determined and requires further research.

It is clear from the research described above that many of the risk factors for CHD are apparent during childhood and adolescence. Consequently, it is very important to determine if these risk factors can be modified by the use of exercise, as has been found in adults. In Chapter 5 a study is described which examines the relationship between cardiorespiratory fitness and blood lipid-lipoprotein profile in adolescents. The aim of this study was to determine if children with higher cardiorespiratory fitness levels have more favourable blood lipid profiles. The literature in adults suggests that exercise can reduce postprandial lipaemia. There is currently no research investigating whether this is also the case in children and in adolescents. In addition the effect of intermittent games activity on postprandial lipaemia has yet to be determined. Therefore in Chapter 6 the effect of intermittent games activity on postprandial lipaemia in young adults is examined and in Chapter 7 the effect of both continuous exercise and intermittent games activity on postprandial lipaemia in adolescents is examined.

Chapter 3: General Methods

3.1 General Introduction

This chapter describes the methodological procedures and the equipment employed in the experimental studies described in this thesis. All of the laboratory tests were carried out in the laboratories and sports hall facilities of the School of Sport and Exercise Sciences at Loughborough University. The Loughborough University Ethical Advisory Committee approved the methods described in this chapter prior to the commencement of data collection. All procedures were carried out in accordance with the "Code of Practice for Workers having Contact with Body Fluids" and prior to involvement in a study all researchers were checked by the Criminal Records Bureau.

3.2 Participant Recruitment

The adult participants who volunteered to take part in the studies described in this thesis were recruited from within Loughborough University by general advertising (see Appendix A1). The child participants who volunteered to take part came from schools in Loughborough, Leicester, Derby and Rugby and also from various National Governing Body Sports Groups who attended the Nike© Summer Performance Camps at Loughborough University (rugby football league, rugby football union, woman's rugby football union, taekwondo, triathlon and badminton), Nottingham Forest Football Academy and Leicestershire Cricket Academy. All Individuals were given written information explaining the study rationale, the requirements of the study design, the procedures and techniques involved, and any possible risks and discomforts participation in the study could entail. This information was also explained verbally and individuals were encouraged to seek clarification if necessary (see Appendix A2). In the case of child participants this information was also given in writing to their primary caregiver. All participants signed a statement of informed consent or if an individual was under the age of eighteen, informed assent (their primary caregiver was required to give their written informed consent) before any testing took place (see Appendix A3). In addition, each participant was required to complete a health history questionnaire (see Appendix A4) in the presence of an investigator who provided any clarification and assistance that

was needed. Any individual with a known history of cardiovascular or coagulation / bleeding disorders or metabolic disease was excluded from involvement in a study.

3.3 Preliminary Tests

3.3.1 Measurement of Body Height and Mass

Height was measured using a Holtain fixed-wall stadiometer (Crymych, U.K.). Participants were instructed to stand with their heels together against a metal plate in a relaxed, but erect, stance. The investigator ensured the buttocks and the back of the head were in contact with the vertical board of the stadiometer. The head was placed in the Frankfort Plane, with the line between the lower orbits of the eyes and the external auditory meatii (the ear holes) perpendicular to the vertical board. The participants were instructed to inhale deeply and the stadiometer headboard was lowered onto the top of the head with enough pressure to compress the hair. In order to compensate for any shrinkage in the intervertebral discs gentle traction was applied to the mastoid processes and height was then read off the stadiometer scale to the nearest 0.1 cm. Body mass was measured to the nearest 0.1 kg using a balance beam (Model 3306ABV, Avery Industrial Ltd., Leicester, U.K.). Participants were dressed in light clothing, without shoes.

3.3.2 Skinfold Thickness and Circumference Measurements

Measurements of skinfold thickness were made using a Harpenden calliper (John Bull, British Indicators, Ltd., UK) on the left side of the body according to the protocol described by Cameron (1984) at the following sites:

a) Triceps – the mid point between the acromion and the olecranon with the arm bent at a right angle. The measurement was taken with the arm straightened and relaxed.
b) Biceps – on the anterior aspect of the arm at the same midpoint level as previously described for the triceps skinfold. The skinfold was picked up with the participant facing the investigator with his or her left arm hanging relaxed with the palm facing forwards. The measurement was taken at the vertical axis joining the centre of the antecubital fossa and the head of the humerus.
c) Subscapular – immediately below the inferior angle of the scapula. The participant stood with his or her back to the observer with shoulders relaxed

and arms hanging loosely at their side. The skinfold was picked up at an angle laterally and downwards, which followed the oblique muscles.

d) Supra-iliac – picked up vertically 1 cm above and 2 cm medial to the anterior superior iliac spine in the mid-axiliary line.

e) Abdominal – picked up vertically at the level of the umbilicus and 5 cm to the left.

f) Anterior thigh – picked up vertically midway between the mid-inguinal point and the proximal border of the patella.

g) Medial Calf – picked up vertically at the level of the maximum calf circumference on the medial aspect of the calf.

Each skinfold was lifted between the thumb and middle finger of the investigator's left hand, 1 cm above the site of measurement. The skinfold callipers were then applied level with the area marked and the measurement taken when the dial of the calliper came to a halt.

Circumference measurements were measured using a non-stretch tape on the left side of the body according to the protocol described by Cameron (1984) at the following sites:

a) Upper-arm – measured at the same point as the triceps skinfold with the arm hanging loose.

b) Waist – the minimum circumference between the iliac crests and lower ribs.

c) Hip – at the level of greatest protrusion of the buttocks with the participant standing erect with their feet together.

d) Thigh – taken at the point the skinfold was taken with the participant standing with their leg relaxed and all their weight on their right leg.

e) Calf – participant sat on a table/bench such that the investigator could be near to or at eye-level with the calf, which was unsupported. The tape was passed around the limb at the area of greatest bulge of the gastrocnemius muscle. The tape was then tightened and the circumference noted. The investigator then moved the tape either higher or lower and repeated the measurement noting if the new circumference was greater or smaller than the previous one. This procedure was repeated above and below the first level until the maximum circumference was found, which was the measurement taken.

For both the skinfold thickness and circumference measurements the mean of three measurements were taken (except for the calf circumference measurement were the maximum value was taken).

3.3.3 Measurement of Blood Pressure

Blood pressure was measured by auscultation using a mercury sphygmomanometer (Accoson Freestyle, CardioHinetics, Salford, U.K.) and a blood pressure stethoscope (Littman, 3M, U.S.A.) after the participant had been seated for approximately 15 min. Appearance of the first Korotkoff sound was used as a criterion for systolic pressure and the fourth Korotkoff sound (i.e. muffling of the sound) defined diastolic pressure.

3.3.4 Self-Assessment of Sexual Maturity

Each child participant was asked to make a self-assessment of sexual maturity using the five-point scale described by Tanner (1962) to assess the development of breasts in girls, genitalia in boys and pubic hair in both sexes. The scale ranges from 1 (pre-pubescent) to 5 (adult). Participants were asked by an investigator of the same sex to view Tanner's photographs and read the descriptions of the different stages of development during puberty and then to mark with an 'A' the stage which most closely matched their current stage of development and a 'B' the next closest (see Appendix A5). This was performed within an enclosed and lockable room with a full length mirror, with which participants could check their stage of development against the photographs. Participants were reminded that their data was anonymous, confidential and for the use of the study only and of their right to refuse assent.

3 3.5 Multistage Shuttle Run Test

During the multistage shuttle run test [(Ramsbottom *et al*, 1988) modified from the original protocol (Leger & Lambert, 1982)] participants are required to run over a marked 20 m distance, in time to an audible signal ('bleep') generated from a pre-recorded CD (The National Coaching Foundation, Leeds, U.K.), turning at each end of the 20 m. Participants start running at 2.22 $m.s^{-1}$ and the speed increases by 0.14 $m.s^{-1}$ approximately every 60 s thereafter. Participants are required to keep running until they were unable to keep pace with the audible signal. A test ended and peak $\dot{V}O_2$ was regarded as being attained, when a participant withdrew or was unable to

reach the taped line in time with the 'bleep' on three consecutive occasions. Strong verbal encouragement was given throughout the test.

3.3.6 Treadmills

Two different makes of motorised treadmills were used in the studies in this thesis. Runner Galaxy M.J.C., MTC Climb 2000, Bianchiah and Draghetti Sac, Cavezzo, Italy in the studies described in Chapters 5 and 6, and Technogym, Italy in the study described in Chapter 7. Prior to each study in which a treadmill was used, the velocity of the unloaded treadmill belt was checked against the digital reading given by the treadmill display across a range of suitable speeds. The length of the treadmill belt was established prior to these measurements. One hundred revolutions of the treadmill belt were timed and then the actual belt speeds were calculated.

3.3.7 Treadmill Familiarisation

Prior to testing, all child participants and any adult participant unaccustomed to treadmill exercise were familiarised with treadmill walking and running and with testing procedures. Firstly participants were asked to walk on the treadmill until they felt confident and at ease. The speed of the treadmill was then increased to 7 $km.h^{-1}$ and participants jogged at this speed until they felt comfortable. Participants were then given instructions on how to perform an "emergency-stop". Participants were then fitted with a nose-clip and mouthpiece. Finally, participants jogged at a speed of 7 $km.h^{-1}$ and were familiarised with the Rating of Perceived Exertion (RPE) scale (Borg, 1973). They then practiced fitting, removing and passing the nose-clip and mouthpiece to the investigator whilst running. The gradient of the treadmill was then increased to an incline of 3 % so that the participant could experience the gradient increasing, after which the participant performed another emergency-stop. The familiarisations were not of a set duration, they lasted as long as was necessary for participants to feel competent exercising on the treadmill and to become familiar with the procedures.

3.3.8 Submaximal Incremental Treadmill Test

In the study described in Chapter 5 each participant performed a continuous, incremental treadmill test that comprised of 4 min stages of increasing exercise

intensity to determine the submaximal blood lactate and oxygen uptake responses of each participant (see Figure 3.1). Participants began running at a speed of 7.0 $km.h^{-1}$ with no incline. The running speed of each subsequent stage was 8.8, 10.6, 12.6, 14.2 $km.h^{-1}$, respectively (a few individuals also completed an additional stage at 16.4 $km.h^{-1}$). It was expected that the participants would have a wide range of running abilities. Consequently, it was necessary to have some slow running speeds at the beginning of the test and some quick running speeds at the end. There are uneven increments in running speeds in the middle of the test in order that the more able runners were not required to run an excessive number of stages. The number of stages completed was dependent on the participant's ability and ranged from two to six stages. Heart rate ($beats.min^{-1}$) was monitored and noted during the last minute of each stage using short-range telemetry (Polar 8810, Finland). Expired air samples were also collected during the last minute of each stage using the Douglas bag method. Participants breathed through a low resistance respiratory valve and 30 mm wide bore, low resistance tubing (Falconia flexible ducting, Baxter, Woodhouse and Taylor Ltd., Macclesfield, U K.) into previously evacuated 150 litre capacity Douglas bags (Plysu Protection Systems, Milton Keynes, U.K.). The percentage of oxygen and carbon dioxide in each of the expired air samples was measured using a paramagnetic oxygen analyser and an infrared carbon dioxide analyser (Servomex, Crowborough, East Sussex, U.K.). Each analyser was calibrated before each experiment using certified reference (nitrogen / oxygen-carbon dioxide) gases (CryoService Ltd , Worcester, U K.). The volume and temperature of the expired air was determined using a dry gas meter (Harvard Apparatus, Edenbridge, Kent, U.K.), which had been fitted with an electronic thermistor and logger (Edale Instruments Ltd., type 2984, Model C). Barometric pressure was established using a Fortin barometer (F. D. and Company, Watford, U.K.). Inspired gas volumes were calculated using the Haldane transformation, and oxygen uptake, carbon dioxide, minute ventilation and respiratory exchange ratio were all subsequently calculated. All gas volumes were corrected to standard temperature and pressure for a dry gas (STPD).

The participant's RPE was noted mid-way though each collection period. At the same time as the air sample was being collected participants placed a hand lightly on one of the treadmill safety rails and duplicate capillary blood samples were collected from one of their fingers (if a participant only completed two stages of the test their blood

lactate concentrates were not used in the final analysis). The test was halted when the investigator deemed the subject to be exercising close to, but not at, maximal capacity. This was determined by examining RPE and heart rate values. Participants were generally stopped if their RPE rating was either 17 or 18 and heart rate was between 170 and 190 beats.min^{-1}.

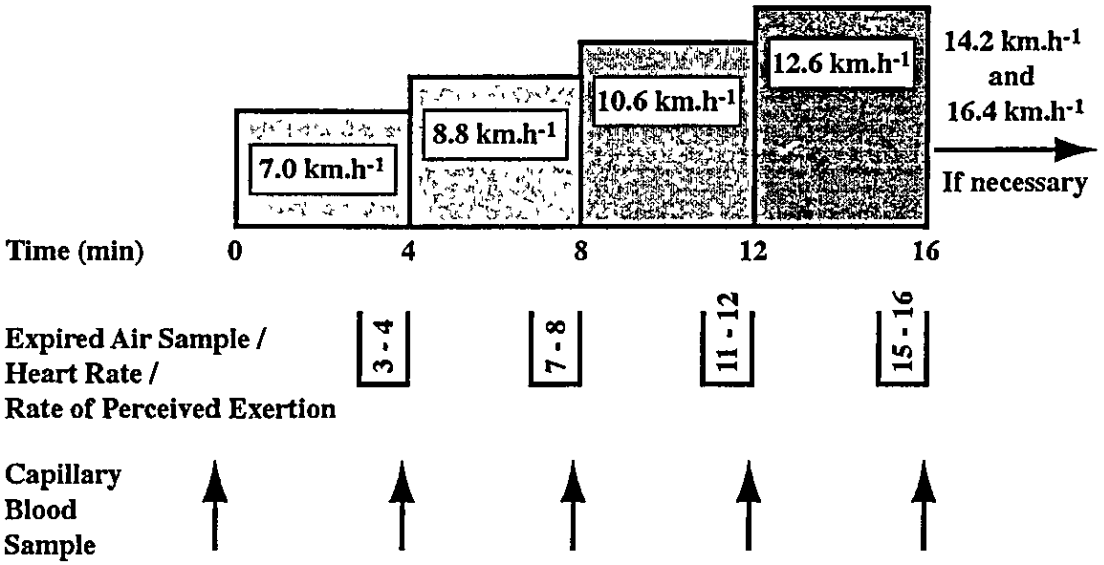

Figure 3.1 Submaximal, incremental treadmill test protocol used in study described in Chapter 5

3.3.9 Peak Oxygen Uptake Test

In the study described in Chapter 5 peak oxygen uptake (peak $\dot{V}O_2$) was determined using a continuous progressive, incremental uphill running treadmill test to voluntary exhaustion. The test protocol commenced at a belt speed chosen by the investigator that elicited a heart rate of approximately 170 beats.min^{-1} during the submaximal treadmill test and at an initial incline of 3 % (the aim being to achieve voluntary exhaustion in 8 – 12 min). The belt-speed remained the same throughout the test with the inclination increasing by 2 % at the end of every 3 min stage. Heart rate (Polar 8810, Finland) was monitored and RPE (Borg, 1973) noted between 1 min 45 s and 2 min 45 s of each stage. Expired air samples were also collected during this time using the Douglas bag method and analysed as described in Section 3.3.8. Participants kept running until they could exercise for 60 s and no longer. When they indicated they had reached that stage a final expired air collection was made and the test ended (see Figure 3.2). Strong verbal encouragement was given throughout the test. The highest oxygen uptake value achieved during the test (usually the final collection) was accepted to be the participant's peak $\dot{V}O_2$.

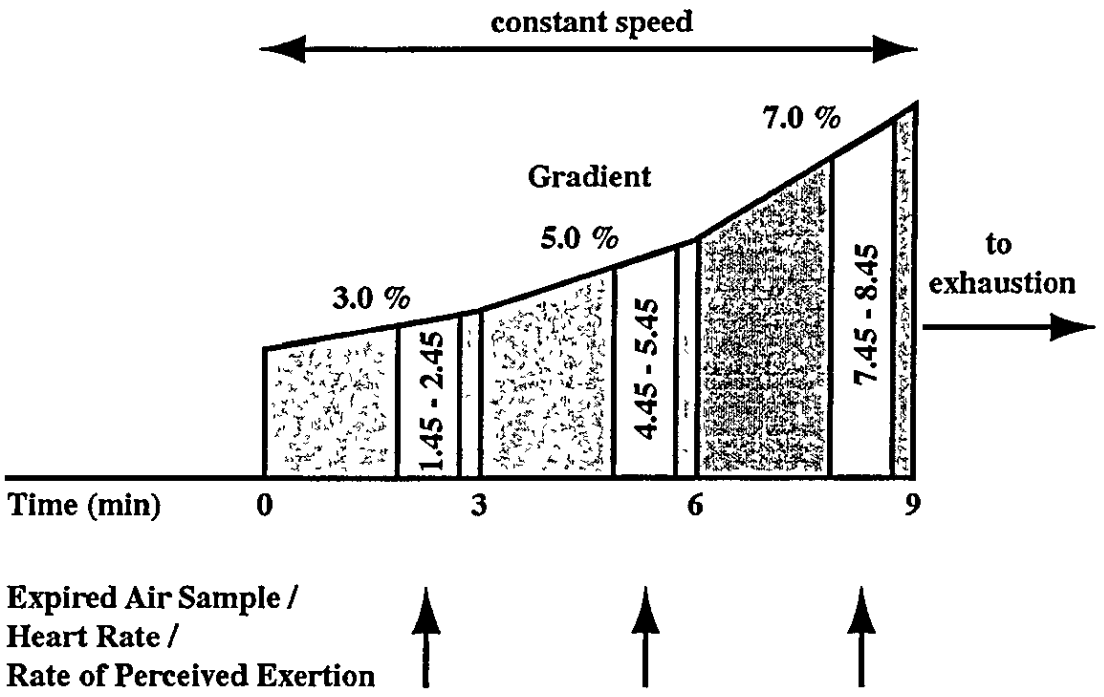

Figure 3.2 Peak oxygen uptake protocol used in the study described in Chapter 5

In Chapters 6 and 7 peak $\dot{V}O_2$ was determined using a protocol that was different from that used in Chapter 5. A continuous progressive, incremental uphill walking treadmill test to voluntary exhaustion was used as this test was also used to set the speeds and gradients that the participants walked at during the walk main trials. The test protocol commenced at a belt speed selected by the participant and at an initial incline of 5 %. The belt-speed remained constant throughout the test with the inclination increasing by 4 % at the end of every 3 min stage. Heart rate (Polar 8810, Finland) was monitored and RPE (Borg, 1973) noted between 1 min 45 s and 2 min 45 s of each stage. Expired air samples were also collected during this time using the Douglas bag method and analysed as described in Section 3.3.8. In addition duplicate capillary blood samples were collected at rest, and during the expired air collections in each 3 min stage for lactate determination. Participants kept running until they could exercise for 60 s and no longer. When they indicated they had reached that stage a final expired air collection was made and the test ended. A final blood sample was then collected. Strong verbal encouragement was given throughout the test. The highest oxygen uptake value achieved during the test (usually the final collection) was accepted to be the participant's peak $\dot{V}O_2$.

3.3.10 Familiarisation with the Main Trial Exercise Protocols in Chapters 6 and 7

In the studies described in Chapters 6 and 7 participants were familiarised with the Loughborough Intermittent Shuttle Test (LIST) and the walk protocols. The LIST was used to simulate games activity and was a modified version of the test reported by Nicholas and colleagues (2000). The main trial protocol is described in detail in Section 3 4.2 and shown in Figure 3.4. The familiarisation sessions required participants to perform four blocks of the uphill walking protocol or the LIST (participants performed 2 – 4 blocks of the LIST in the study described in Chapter 7). This replicated the amount of exercise performed during the main trials. These sessions were used to set and check the exercise intensity for the main trials.

3.4 Main Trial Protocol, Procedures and Measurements in Chapters 6 and 7

3.4.1 Main Trial Protocol

In the study described in Chapter 6 each participant took part in three main trials: a Rest trial, a Walk exercise trial and a LIST exercise trial a minimum of 6 days apart, in a balanced cross-over design (see Figure 3.3). In the study described in Chapter 7 each participant took part in two main trials: a Rest trial and either a Walk exercise trial or a LIST exercise trial. During the Rest trial participants refrained from exercise on day one of the experiment and then performed an oral fat tolerance test on the morning of day two. On the afternoon of day one during the exercise trials, participants performed a 15 min warm-up on a treadmill consisting of 7 min of walking at a 1% gradient, at the speed maintained during the peak $\dot{V}O_2$ test, 5 min of stretching and 3 min of walking at a speed and gradient selected to elicit 60% of peak $\dot{V}O_2$. Participants then completed either four blocks of treadmill walking or four blocks of the LIST, with 3 min rest between each block. The next morning (day two) participants attended the laboratory for a fat tolerance test. Exercise was completed at the same time of day (between 3:30 and 5 pm) in both exercise trials so that the time interval between the end of the exercise sessions and beginning of the fat tolerance tests was similar in both exercise trials ($\approx$16 h).

Participants were asked to refrain from physical activity for the 2 days before the commencement of each trial and also to weigh and record their food and drink intake during the two day period before the oral fat tolerance test of their first trial

(participants involved in the study described in Chapter 6 were asked to record their food and drink intake and make note of portion size). They were then asked to replicated this diet for all subsequent trials. Participants were also asked not to consume alcohol during these periods.

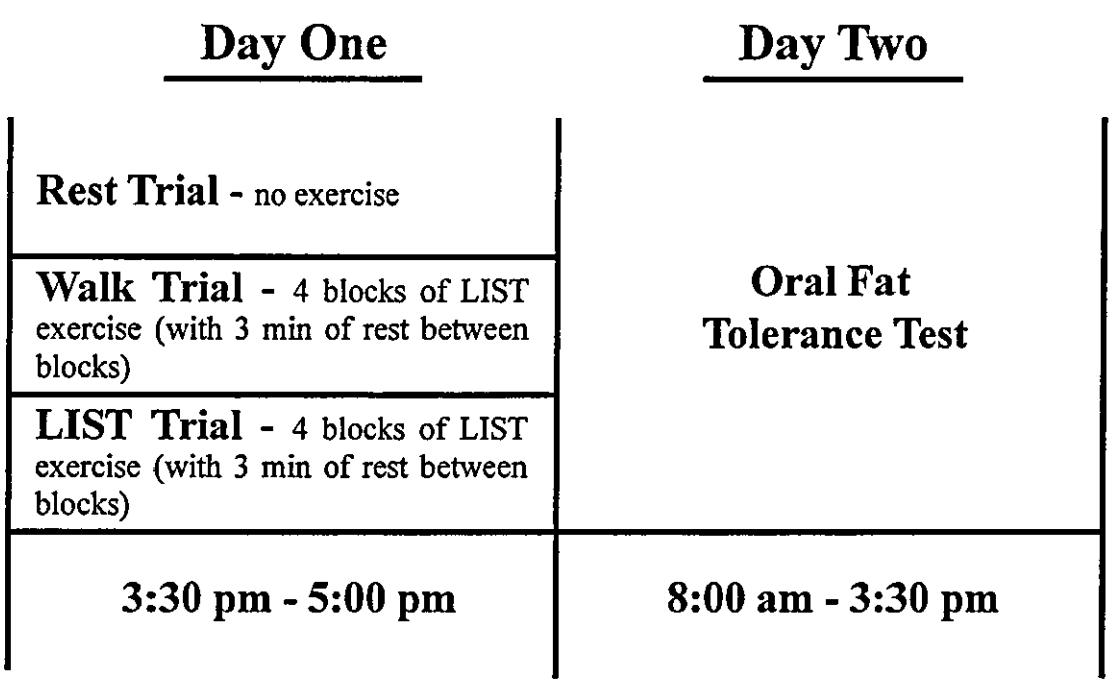

Figure 3.3 Study protocol. Three trials each over two days: day one – either Walk or LIST session or activities of daily living (rest), day two – oral fat tolerance test. In the study described in Chapter 7 participants completed two trials a rest trial and either a Walk or LIST trial

3.4.2 Main Trial Exercise Protocol

Both the walk and LIST exercise sessions consisted of four blocks of exercise, with 3 min rest periods between each block. An attempt was made to set the exercise intensity for both exercise modes at an average of 60% $\dot{V}O_2$ peak over each block.

During the walk sessions in Chapter 6 three expired air collections were made during the 11:00 – 12:00, 12:30 – 13:30, and 14:00 – 15:00 min of each block. The first two collections in each block were made using the standard Douglas bag method and the last collection using a Douglas bag attached to a rucksack. Participants were given the rucksack to wear approximately 30 s before the first collection. The three collections were made in order to determine whether there were any differences

between measurements made using the standard Douglas bag method and those made using the Douglas bag attached to a rucksack since this was the method used to make expired air collections during the LIST session. During the walk sessions in Chapter 7 expired air collections were made during the last minute of each block (14.00 – 15·00 min) using standard Douglas bag protocol. Heart rate was monitored and RPE noted during each expired air collection. Capillary blood samples were taken to determine blood lactate concentration at rest and immediately after the completion of each block (Chapter 6) or at rest and on the completion of block 1 and 4 (Chapter 7).

A diagrammatic representation of the LIST protocol is presented in Figure 3.4. The LIST sessions took place in a sports hall on a flat, non-slippery, wooden floor. Participants completed ten cycles of the walk, sprint, run ('cruise') and 'jog' pattern of exercise over a marked 20 m distance, which formed one "block" of exercise. Participants were allowed 13 s to complete each of the three 20 m walks, the sprint was maximal, and the intensity of the cruise and jog phases of the test was set relative to an individuals peak $\dot{V}O_2$ as estimated on the progressive multistage shuttle run test. The average intensities of the cruise and jog phases in the studies were set at 70/40% of estimated peak $\dot{V}O_2$. If the desired exercise intensity of an average of 60% $\dot{V}O_2$ peak (determined using oxygen uptake values measured during rest periods) over each block was not achieved using the running speeds predicted from the multistage shuttle run test, the speeds were lowered until the intensity achieved was as close as possible to 60% of $\dot{V}O_2$ peak, without the speeds becoming so slow participants had to walk.

Participants were able to gauge their required running speeds and exercise intensity by following amplified audio signals generated by a computer. Investigators provided participants with information about the type of exercise required during the next 20 m (such as "walk", "sprint", "cruise" or "jog"), ensured subjects kept pace with the audio signals from the computer and reminded them immediately prior to each sprint to perform it maximally. Fifteen metre sprint times were measured using 2 infrared photoelectric cells (RS Components Ltd., Corby, UK) connected to a computer.

During the LIST sessions an expired air collection was made during the 10^{th} cycle of each block using a Douglas bag attached to a rucksack. Heart rate was monitored throughout the exercise sessions (Polar Electro S810, Finland) and RPE was noted

just before sprint 8 of each block Capillary blood samples were collected as in the walk sessions (see above).

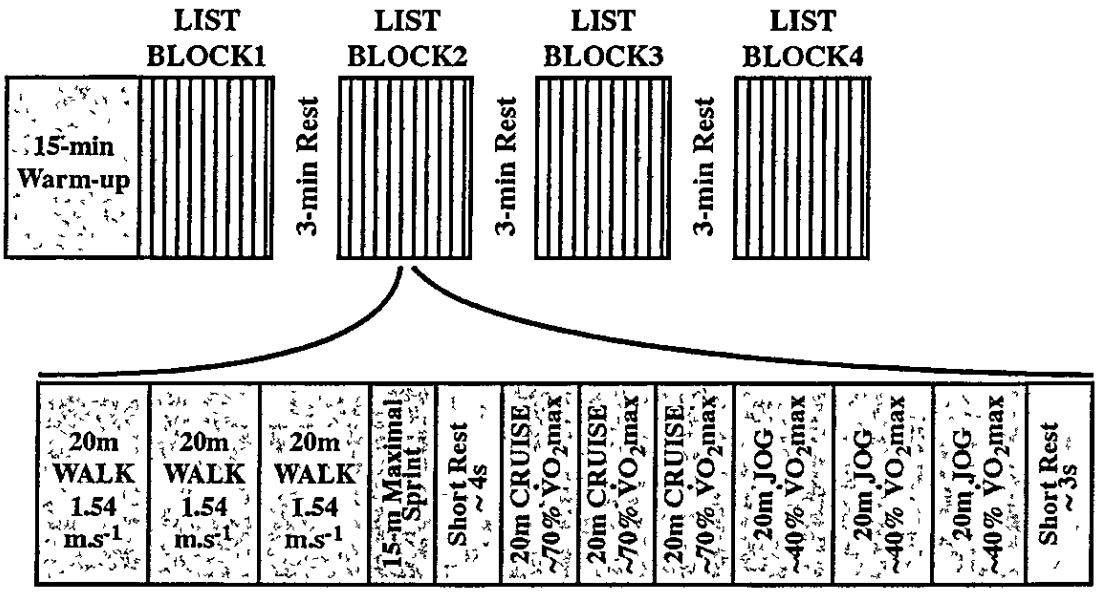

Figure 3.4 The LIST protocol

3.4.3 Oral Fat Tolerance Tests

A diagrammatic representation of the oral fat tolerance test is presented in Figure 3.5. Participants reported to the laboratory after an overnight fast of at least 12 h, having travelled by car to ensure that they were in a rested state. During the study described in Chapter 6 blood samples collected during the oral fat tolerance test were collected with the aid of a cannula, therefore a cannula was introduced into an antecubital or forearm vein at this point of the study (see Section 3.5). Participants rested quietly while their resting metabolic rate was measured for 15 min (see Section 3.4.5). A baseline blood sample was then taken (see Section 3.5). A high fat meal (see Section 3.4.4) was then ingested. Further blood samples were taken 0.5, 0.75 and 1 h, and then hourly during a 6 h period of observation (Chapter 6) or at 0.5, 0.75, 1, 3, 4 and 6 h (Chapter 7). Postprandial metabolic rate was measured at 2.5 and 5.5 h. Participants rested or performed sedentary activities (watched television and videos, played video games and read) during the 6 h postprandial observation period. No food or drink other than water was consumed during this time. Water intake was *ad libitum* for the first trial and intake was recorded and replicated in subsequent trials. At the end of the observation period if a cannula had been used during the trial it was removed and haemostasis ensured before the participant left the laboratory.

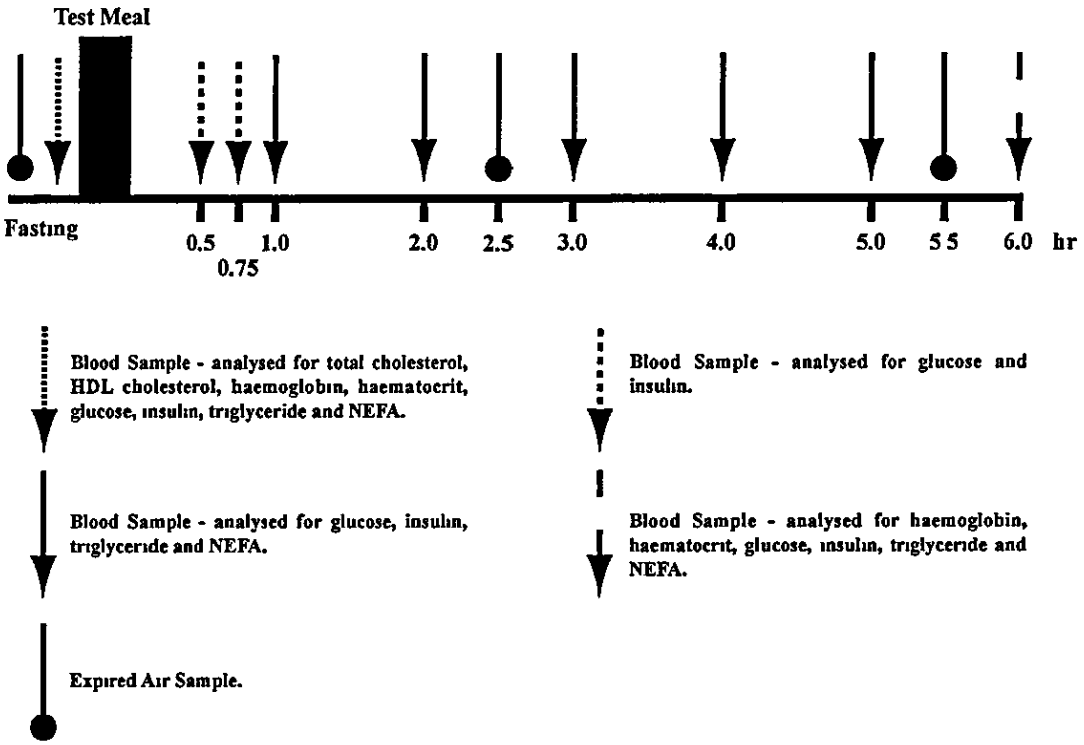

Figure 3.5 The Oral fat tolerance test protocol. In the study described in Chapter 7 blood samples were not collected at 2 and 5 h or analysed for insulin or NEFA.

3.4.4 High Fat Test Meal

The test meal employed in the studies of this thesis was a high fat, mixed meal and a modification of the meal described previously by Schlierf and colleagues (1987). It comprised of cereal, fruit, nuts, chocolate and whipping cream, with the whipping cream being the main contributor to the fat load. It was given according to body mass (see Table 3.1) and its macronutrient composition is shown in Table 3.2. The reproducibility of this test meal has been examined by Gill & Hardman (1998) and found to have enough precision to detect exercise-induced decreases in postprandial lipaemia.

Table 3.1 Quantities of ingredients of high fat test meal, prescribed according to participants body mass

Ingredient	Quantity ($g.kg^{-1}$ body mass)
Whipping cream	2.531
Apple	0.670
Banana	1.135
Milk chocolate	0.134
Sultanas	0.134
Brazil nuts	0.147
Oats	0.750
Desiccated coconut	0.067

Table 3.2 Macronutrient composition of high fat meal

	Quantity ($g.kg^{-1}$ body mass)
Energy (kcal)	16.04
Fat (g)	1.25
Carbohydrate (g)	1.07
Protein (g)	0.20
Energy from fat %	70
Energy from carbohydrate %	25
Energy from protein %	5

3.4.5 Measuring Energy Expenditure at Rest and During The Postprandial Period

In the study described in Chapter 6 resting metabolic rate and postprandial energy expenditure were measured using a ventilated hood attached to an automated metabolic cart (GEM Europa Scientific, NutrEn Technology Ltd., Manchester, U.K.). Participants spent 15 min under the hood. The first 5 min served as a habituation and calibration period. Oxygen consumption and carbon dioxide production were averaged every 30 s for the remaining 10 min. In the study described in Chapter 7 resting metabolic rate and postprandial energy expenditure was measured by collecting 5 min expired samples using the standard Douglas bag protocol. This method was changed from that used in Chapter 6 as 4 participants were general measured at once and only one ventilated hood was available.

3.5 Blood Collection, Treatment, Storage

Capillary blood samples were collected from the finger of participants during the submaximal incremental treadmill test (see Section 3.3.8), the peak $\dot{V}O_2$ test in the studies described in Chapters 5 and 7 (see Section 3.3.9) and during the Walk and LIST exercise sessions (Section 3.4.2). An automatic lancet (Accu-Check, Softclix Pro, Roche Diagnostics Ltd , Lewes, U.K.) was used to pierce the skin and duplicate 20 µl volumes of blood were collected into non-heparinized micro pipettes (Accupette Pipettes, Baxter Healthcare Corporation, U.S.A.). These volumes were then immediately deproteinised in microcentrifuge tubes (Sarstedt Ltd., Leicester, U.K.) containing 200 µl of cold 0.4 mol l^{-1} (2.5 %) perchloric acid. The samples were then centrifuged (Eppendorf-Anderman Centrifuge 5414, Germany) at 13000 rev.min^{-1} for 3 min and stored at -20 °C for subsequent analysis of lactate concentration (Section 3.6.4).

In the study described in Chapter 6 venous blood samples were obtained during the oral fat tolerance test via an indwelling cannula (18 gauge / 45 mm cannula, Venflon 2, BOC Ohemeda AB, Helsingborg, Sweden), inserted into an anticubital or forearm vein under local anaesthetic (Lignocaine hydrochloride 1 % w/v, Antigen pharmaceuticals Ltd., Roscrea, Ireland). The cannula was connected to a three-way stopcock (Connecta, BOC Ohmeda AB, Helsingborg, Sweden). A sterile, non-heparinised saline solution (B. Braun Medical Ltd., Buckinghamshire, U.K.) was used to keep the cannula patent. Five (0.5 and 0.75 h) or 10 ml (fasting and hourly) samples of venous blood were drawn into appropriately sized syringes (Becton-Dickinson, Oxford, U.K.) and the blood was immediately dispensed into pre-chilled potassium ethylenediamine tetraactic acid-coated (EDTA) collection tubes (Sarstedt Ltd., Leicester, U.K.). All samples were taken after the participant had been in the supine position for at least 10 min as changes in posture may influence plasma volume (Rowell, 1993). Small volumes of venous blood were sampled from the tubes for haemoglobin and haematocrit determination (see Section 3.6.1). Blood samples were then centrifuged (Koolspin, Burkard Scientific Ltd., Uxbridge, U.K.) at 4000 revs.min^{-1} for 10 min at 4 °C. The plasma was then transferred into a plain tube and then inverted to mix. It was then dispensed into microcentrifuge tubes (Sarstedt Ltd., Leicester, U.K.) in aliquots of not less than 0.5 ml to minimise any freeze-drying effect and stored at – 80 °C, for later analysis.

In the study described in Chapter 7 capillary blood samples were taken during the oral fat tolerance test. To minimise postural variations in lipoprotein values, participants were seated for at least 10 min before samples were taken. When a high volume of blood was needed the participant's hand was immersed in warm water to increase superficial blood flow and so arterialise the capillary blood. The skin was punctured using an automatic lancet (Accu-Check, Softclix Pro, Roche Diagnostics Ltd., Lewes, U.K.) and the required amount of blood was collected into the appropriate tubes (i.e. 2 x 20 µl non-heparinised micro-pipettes (Accupette Pipettes, Baxter Healthcare Corporation, U.S.A.) for haemoglobin analysis, 2 x 20 µl non-heparinised micro-pipettes (Accupette Pipettes, Baxter Healthcare Corporation, U.S.A.) for glucose analysis, 2 x 30 µl heparinised micro-haematocrit tubes (Scientific Industries, Loughborough, UK) for haematocrit analysis, 1 x 30 µl Reflotron capillary tubes (Reflotron/Lipotrend C 30 µl, Roche) for total cholesterol, and 1 x microvette CB 300 tubes (Sarstedt Ltd, Leicester, UK) for HDL and TAG analysis.

For glucose analysis the capillary whole blood samples collected were immediately deproteinised in microcentrifuge tubes (Sarstedt Ltd., Leicester, U.K.) containing 200 µl of cold 0.4 mol^{-1} (2.5 %) perchloric acid. The samples were then centrifuged (Eppendorf-Anderman Centrifuge 5414, Germany) at 13000 $rev.min^{-1}$ for 3 min, and stored at -20 °C for subsequent analysis. The capillary whole blood collected for HDL and TAG analysis, was centrifuged (Eppendorf-Anderman Centrifuge 5414, Germany) at 13000 $rev.min^{-1}$ for 3 min, 30 µl of plasma was then removed with a pipette (Gilson Pipetman, Gilson Medical Electronics, France) for HDL cholesterol analysis using the Reflotron, in addition 2 x 20 µl of plasma was removed and then diluted in duplicate 50 times by addition of 980 µl of ice-cold saline (155 $mmol.l^{-1}$) (in order to prevent any freeze-drying effect as a result of storage) and then stored at -20 °C for subsequent analysis.

3.6 Analysis of Blood Samples

All analyses were carried out in the Biochemistry Laboratory of the School of Sports and Exercise Sciences at Loughborough University unless otherwise stated.

3.6.1 Estimation of Changes in Plasma Volume

Haematocrit was determined in triplicate (Chapter 6) or in duplicate (Chapter 7). Venous (Chapter 6) or capillary (Chapter 7) blood was collected in small bore tubes and these were then centrifuged for 15 min (micro- haematocrit centrifuge, Hawksely and Sons Ltd, Lancing, U.K.). The haematocrit portion of the blood sample was subsequently established using a micro-haematocrit reader (Hawksely and Sons Ltd., Lancing, U.K.). In addition, haemoglobin concentration was established spectrophotometrically (Shimadzu Spectrophotometer, Shimadzu Corporation, Kyoto, Japan), in duplicate 20 µl volumes of blood using the cyanmethaemoglobin method. Changes in plasma volume (%) were estimated using the method of Dill and Costill (1974).

3 6.2 Spectrophotometric Assays

In the study described in Chapter 6 plasma samples were analysed for total cholesterol, HDL cholesterol, TAG, glucose (Randox Laboratories Ltd, U.K.) and NEFA (Wako Chemicals GmbH, Germany) by enzymatic, colorimetric methods with the use of a centrifugal analyser (Cobas Mira Plus, Roche, Basel, Switzerland). In the study described in Chapter 7 the plasma samples which where diluted 50 times with saline were analysed for TAG using the same method. Capillary whole blood glucose concentration was determined spectrophotometrically (Shimadzu Spectrophotometer, Shimadzu Corporation, Kyoto, Japan) using a commercially available kit (Randox Laboratories Ltd; UK) as described by Maughan (1982).

3.6.3 Radioimmunoassays

In the study described in Chapter 6 plasma samples were analysed for insulin using a solid-phase 125 Iodine radioimmunoassay available in a commercial kit (ICN Pharmaceuticals, Inc., Costa Mesa CA U.S.A.). Radioactivity was measured using an automated gamma counting system (Cobra II, Packard Instrument, Downers Grove, IL U.S.A.). This analysis was conducted in the Radiochemistry Laboratory, in the Chemistry Department at Loughborough University.

3 6.4 Fluorimetric Assays

In all studies blood lactate concentration was established using an enzymatic, fluorimetric method described by Maughan (1982). A Locarte Fluorimeter (Model 8-9, Locarte, London, U K.) or a Spectrofluorimeter (RF-1501, Shimazu, Japan) was used to measure fluorescence.

3.6.5 Dry Chemistry Analysis Using The Reflotron

The Reflotron is a microprocessor-controlled reflectance photometric analyser using dry chemistry reagents for discrete analyses and reflectance photometry to evaluate the blood samples. Reagent strips, which are pre-calibrated by the manufacturer, are used with the analyser. Within 5 min of collection the capillary blood, which was collected in the Reflotron capillary tubes was dispensed using the Reflotron pipette, onto the total cholesterol reagent strips for total cholesterol measurement. A calibrated precision micro litre pipette (Gilson, France) was used to dispense a 30µl plasma sample onto the HDL cholesterol reagent strip. Within 15 s of the sample being applied and absorbed, each strip was inserted into the Reflotron instrument for reading. In the Reflotron, the sample is absorbed through a glass filter layer, which separates the plasma from the cells. The heparinised whole blood (for total cholesterol) and EDTA treated plasma (for HDL cholesterol) then moves into a zone impregnated with reagents and enzymes necessary for the measurement. After a specific time period (175 s for total cholesterol and 90 s for HDL cholesterol), the instrument measures the reflectance (colour intensity) of the sample and displays the measurement digitally in concentration ($mmol.l^{-1}$).

3.7 Accuracy and Precision of Assays

The accuracy of the assays described in the sections above was monitored using quality control sera (Lactate Control (Randox Lipid Control Levels I, II, III, (Randox Laboratories Ltd, U K.), Seronorm Lipid (Sero AS, Billingstad, Norway), Precinorm HDL and Precinorm U (Roche Diagnostics, Lewes E. Sussex, U.K.)). All samples for each individual participant were performed in the same analyser run for each assay (except samples measured using the Reflotron and for haematocrit and haemoglobin as they were analysed on each testing day). Within-batch coefficients of variations were 1.3% for TAG, 2.6% for glucose, 1.9% for total cholesterol, 0.9% for HDL

cholesterol, 1.1% for NEFA, 9.7% for Insulin, 1.3% for lactate. For the Reflotron analyser the coefficients of variations were 2.5% for glucose, 0.8% for total cholesterol and 4.5% for HDL cholesterol.

3.8 Statistical Analysis

A variety of statistical procedures were used to analyse the results presented in the studies in this thesis. A detailed description of the procedures used in each study is given in the appropriate chapter. Data are presented as mean and standard error of the mean (SEM), and are based on the subject populations stated. Data were analysed in Chapters 5 and 6 using the Statistical Package for the Social Science (SPSS) software version 11.0 for windows (SPSS Inc, Chicago, IL) and in Chapter 4 using Minitab.

Chapter 4: Preliminary Work

4.1 Introduction

In order to be confident of the measurements made in the studies described in Chapter 5, 6 and 7 it was necessary to investigate the reliability and criterion validity of some of the techniques used in these studies.

The term reliability is often used interchangeably with others such as repeatability, reproducibility, consistency and agreement (Atkinson & Nevill, 1998). Bland (2000) argues that essentially any physiological measurement will have natural biological variability as a result of changes within the system, and also that there will be errors associated with the method chosen to assess the particular variable. For example blood pressure will vary from season to season, day to day and even heart beat to heart beat. The perceptions of Korotkoff sounds of the individual establishing the blood pressure, and of the reading on the manometer, will also vary from measurement to measurement. Some means to quantify this variation or measurement error is important. A number of different methods have been used to assess reliability among them the correlation coefficient, the t-test and more recently limits of agreement (Bland & Altman, 1986) and typical error (Hopkins, 2000). In the analysis and discussion that follows a 'belt and braces' approach has been followed in that a number of different procedures have been used to assess reliability. This is because there does not seem to be any generally agreed definitive method for establishing reliability and a variety of methods have been used in previous research literature (Atkinson & Nevill, 1998).

Closely linked to the idea of reliability is the concept of criterion validity; that is the extent to which a tool agrees with previous methods used to measure the same variable (Strand & Wilson, 1993). This concept is of importance because on many occasions when physiological measurements are made they are actually indirect assessments of a variable as it may not be possible to make a direct assessment. Therefore one is often comparing one indirect method of measurement with another (Bland, 2000).

4.2 Comparison of Two Different Analysers (Reflotron and Cobas Mira Plus) When Used to Determine Postprandial Lipoprotein Concentrations in Venous and Capillary Blood.

4.2.1 Introduction

Most humans eat some food 4-6 times per day and therefore spend most of their waking hours in the postprandial state. Postprandial lipid and lipoprotein profiles can provide a measure of an individuals coronary heart disease risk (Zilversmit, 1979). Exaggerated postprandial lipaemia indicates poor TAG metabolic capacity (Patsch *et al.*, 1992) and has been implicated in atherosclerosis, a process that is believed to begin in childhood. There have been many studies investigating postprandial lipaemia in adults, but not in children.

There are a number of reasons why the often invasive methods and procedures used in investigations where adults are the participants may be inappropriate for use in studies involving children and adolescents. The age, experience and incomplete emotional and physical development of children and adolescents means that researchers have a moral and ethical duty of care not to put young participants in a study at unnecessary risk. Also, from a practical point of view the use of invasive procedures in an investigation might make it unlikely many children and adolescents would assent, or their parents give consent, to involvement in a study

Having considered these issues it was decided that where young people were involved in studies (see Chapters 5 and 7), an automated analyser (Reflotron, Boehringer Mannheim) and finger prick blood sampling would be used to collect and analyse resting and postprandial blood responses. Potentially, finger prick blood sampling may result in different values compared with samples collected via cannula due to differences between capillary and venous blood (Bachorik *et al.*, 1990; Greenland *et al.*, 1990) and due to differences between the analysers used i.e. the Reflotron which is used to determine lipoprotein concentrations from capillary blood versus the Cobas Mira Plus (Roche, Basel Switzerland) which is currently used in our laboratory to determine lipoprotein concentrations from venous blood.

Therefore, the aim of this investigation was to compare postprandial concentrations of total cholesterol, TAG and HDL cholesterol determined by the Reflotron and Cobas Mira Plus analysers.

4 2.2 Subjects, Methods and Statistical Analysis

Following ethical approval and informed consent 10 male subjects (age: 23.5 ± 11.4 years; body mass: 75.5 ± 37.5 kg) completed a 6 h oral fat tolerance test (see Section 3.4.3). The test was undertaken after an overnight fast. Venous blood samples were collected during the oral fat tolerance test from an indwelling cannula. When a venous blood sample was drawn a concomitant capillary blood sample was also taken by finger prick (skin puncture). All blood samples were obtained with the subjects in the same position (lying on a couch) as shifts in plasma volume may occur with changes in posture (Rowell, 1993). Samples were analysed as described in Section 3.5.

Comparisons were made between three methods (Cobas venous plasma (CBVP); Reflotron-capillary whole blood (RFCW); Reflotron venous whole blood (RFVW)) of establishing the concentrations of TAG, glucose and cholesterol, and three methods (Cobas venous plasma (CBVP); Reflotron-capillary plasma (RFCP); Reflotron venous plasma (RFVP)) of establishing HDL-cholesterol, using a variety of statistical procedures: Pearson Correlation coefficient, t-test for correlated samples, and limits of agreement (Bland & Altman, 1986). Data are presented as mean and standard deviation (SD), and are based on the sample numbers stated.

4.2 3 Results

The results of the comparisons between the three measurements methods for each of the blood variables are shown in Tables 4.1 to 4.4.

Table 4.1 TAG concentrations (mmol.l^{-1}) measured using a variety of methods

TAG	COBAS venous (CBVP)	REFLOTRON capillary (RFCW)	REFLOTRON venous (RFVW)
Mean	1.42	1.46	1.45
SD	0.40	0.45	0.45
	$n = 48$	$n = 47$	$n = 45$
	CBVP vs RFCW	**RFCW vs RFVW**	**CBVP vs RFVW**
Pearson r	$r = 0.86$; $P < 0.001$	$r = 0.95$; $P < 0.001$	$r = 0.90$; $P < 0.001$
t-test	$P = 0.403$	$P = 0.131$	$P = 0.921$
bias	-0.03	0.03	0.00
SD of difference	0.24	0.13	0 20
LOA-	-0.49	-0.23	-0.39
LOA+	0.43	0.30	0.38
	$n = 47$	$n = 43$	$n = 44$

LOA = limits of agreement

Table 4.2 Glucose concentrations (mmol.l^{-1}) measured using a variety of methods

Glucose	COBAS venous (CBVP)	REFLOTRON capillary (RFCW)	REFLOTRON venous (RFVW)
Mean	5.40	5.13	4 83
SD	0.79	0.55	0 60
	$n = 79$	$n = 78$	$n = 79$
	CBVP vs RFCW	**RFCW vs RFVW**	**CBVP vs RFVW**
Pearson r	$r = 0.67$; $P < 0$ 001	$r = 0.77$; $P < 0.001$	$r = 0.80$; $P < 0.001$
t-test	$P < 0.001$	$P < 0.001$	$P < 0.001$
bias	0.28	0.30	0.57
SD of difference	0.58	0.40	0 47
LOA-	-0.85	-0.47	-0.35
LOA+	1.41	1.08	1.48
	$n = 78$	$n = 78$	$n = 79$

LOA = limits of agreement

Table 4.3 HDL cholesterol concentrations (mmol.l^{-1}) measured using a variety of methods

HDL cholesterol	COBAS venous (CBVP)	REFLOTRON capillary (RFCP)	REFLOTRON venous (RFVP)
Mean	1.29	1.10	1.08
SD	0.25	0.29	0.26
	$n = 32$	$n = 32$	$n = 32$
	CBVP vs RFCP	**RFCP vs RFVP**	**CBVP vs RFVP**
Pearson r	$r = 0.65$; $P < 0.001$	$r = 0.86$; $P < 0.001$	$r = 0.67$; $P < 0.001$
t-test	$P < 0.001$	$P = 0.584$	$P < 0.001$
bias	0.19	0.01	0.21
SD of difference	0.23	0.15	0.21
LOA-	-0.26	-0.28	-0.20
LOA+	0.64	0.31	0.62
	$n = 32$	$n = 32$	$n = 32$

LOA = limits of agreement

Table 4.4 Total cholesterol concentrations (mmol.l^{-1}) measured using a variety of methods

Total cholesterol	COBAS venous (CBVP)	REFLOTRON capillary (RFCW)	REFLOTRON venous (RFVW)
Mean	4.11	3.56	3.55
SD	0.84	0.67	0.65
	$n = 29$	$n = 29$	$n = 29$
	CBVP vs RFCW	**RFCW vs RFVW**	**CBVP vs RFVW**
Pearson r	$r = 0.96$; $P < 0.001$	$r = 0.99$; $P < 0.001$	$r = 0.95$; $P < 0.001$
t-test	$P < 0.001$	$P = 0.538$	$P < 0.001$
bias	0.55	0.01	0.56
SD of difference	0.27	0.11	0.30
LOA-	-0.02	-0.20	-0.03
LOA+	1.08	0.23	1.16
	$n = 29$	$n = 29$	$n = 29$

LOA = limits of agreement

4.2.4 Discussion

The mean concentrations of TAG were very similar when the three measurement methods were compared and no significant differences were evident from the t-test comparisons (see Table 4.4). The relationships between the three measurement methods were also high (r = 0.86, 0.95 and 0.90 for CBVP vs RFCW, RFCW vs RFVW and CBVP vs RFVW respectively; all $P < 0.001$). However, the Bland and Altman (1986) analysis suggested that, while there was little or no bias when the methods were compared, measured concentrations could vary by as much as ± 0.48 mmol l^{-1}, which represents approximately 33% of the average concentration measured It should also be noted that the Reflotron was unable to measure TAG concentrations below 0.80 mmol.l^{-1}. Fifty percent of the samples collected from the participants in this study for TAG analysis were below this measurement range so could not be used for statistical analysis.

Little information is available concerning the Reflotron's ability to measure TAG. A comparison of TAG measured in capillary whole blood (Reflotron) with venous serum (laboratory method) found a strong relationship (r = 0.97) but concentrations were 10% lower using the Reflotron (von Schenck *et al.*, 1987). This is in contrast with results in this study which found that TAG concentrations measured from capillary whole blood using the Reflotron were on average 2% higher than those measured in venous plasma using the Cobas Mira Plus.

While the correlation coefficients were also high for the glucose concentrations measured by the three different methods, mean values and significant t-test analyses all suggested considerable disagreement between the methods. The Bland and Altman (1986) analysis suggested bias and that measured glucose concentrations could vary by as much as 1.2 mmol.l^{-1} (approximately 25% of the lowest mean value). Similar responses were evident when HDL cholesterol and total cholesterol concentrations were measured, although the level of agreement between the two Reflotron measurements was much better than between either and the Cobas Mira Plus method.

Most of the literature regarding the use of the Reflotron has been based on its use to measure total cholesterol. Its use to measure concentrations of TAG and HDL cholesterol (one of its recently developed tests) has been less well evaluated, and no information regarding its use to determine glucose concentrations has been found. It

is also difficult to compare results between studies because of the considerable variation in the anticoagulants, analysers and blood portions (serum or plasma) used in studies.

In a study performed by Bachorik et al. (1990) total cholesterol concentrations in capillary whole blood were found to be 6% higher than results from venous plasma using a laboratory method (r = 0.9), while other values reported in the literature suggest differences of 4% (Miller *et al.*, 1993) and 3.6% (Greenland *et al.*, 1990) higher. These findings contrast with the results from the present investigation where total cholesterol concentrations were 13.4% lower in capillary whole blood (Reflotron) compared with venous plasma (Cobas Mira Plus, r = 0.96). While changes in the Reflotron technology over time may help to explain the discrepancy it is clear that bias, be it lower or higher is common when two measurement methods are compared.

Several studies have assessed the performance of the HDL cholesterol strip (Ng *et al*, 1991; Warnick *et al.*, 1993). Most of these investigations found the strips to work and there appeared to be little difference in the results whether the blood was obtained from venous or from capillary sources. In a study by Warnick and colleagues (1993) results from capillary whole blood were found to agree well with those from venous plasma not using the Reflotron (r = 0.967), which is much better than that seen in this investigation (r = 0.67). They also found concentrations measured in capillary whole blood to be on average only 4% higher than the venous plasma samples as compared to 14.7% lower in this investigation. It was found by Ng and colleagues (1991) that capillary whole blood values where 1.3% lower than results determined from venous plasma not using the Reflotron (r = 0.999).

Overall, the studies that have evaluated the performance of the Reflotron have not found consistent results. Some have reported positive bias, others negative. It is hard to explain the reasons for these different findings, although it has been suggested that attention should be paid to storage of strips and care taken that the correct sample volume of blood is applied to each strip (Cramb *et al.*, 1990). It is also important that the correct anticoagulant is used for each test. Total cholesterol measurements performed on samples containing EDTA were found to have a bias, while EDTA plasma should be used when measuring HDL cholesterol (Cramb, 1999). Another

potential difficulty is the problem of comparability of venous and capillary blood. Altough Kafonek and colleagues (1996) found no biological variation between lipids determined in capillary samples compared with those in venous samples.

The investigation described above also emphasises the necessity to accurately establish how the concentrations of particular variables have been measured, because it is clear that variations in the use of capillary or whole blood or plasma, and Reflotron or Cobas Mira Plus methods, can considerably influence the concentrations that will be recorded for lipids such as TAG and cholesterol.

Although the mean concentrations of TAG were very similar when the three measurement methods were compared and no significant differences were evident from the t-test comparisons, the Reflotron was found to be unsuitable for measuring TAG concentrations in the postprandial study described in Chapters 6. This was due to its inability to measure TAG concentrations below 0.80 $mmol.l^{-1}$ as it was necessary to measure concentrations below this range. In addition the Reflotron was not used to measure glucose. This was as a result of the high cost of the necessary strips and the availability of a cheaper manual method (Maughan, 1982). It was decided that it was acceptable to use the Reflotron to measure total cholesterol and HDL cholesterol. However it is important to note that concentrations were 13.4% lower for total cholesterol measured in capillary whole blood and 14.7% lower for HDL cholesterol measured in capillary plasma using the Reflotron compared with venous plasma using the Cobas Mira Plus. It is important to consider this if comparing values with other studies.

4.3 Comparison of Oxygen Uptake Measurements Made Using Douglas bags, Carried on the Back Via a Rucksack or Connected to a Rack

4.3.1 Introduction

In Chapter 6 participants performed both continuous uphill treadmill walking or intermittent games activity for approximately 60 min. In Chapter 7 the adolescent participants performed one of these exercise modes. The LIST protocol [adapted from Nicholas et al , (2000)] was used to mirror the demands of intermittent games activity typical of that seen in sports such as hockey, rugby and soccer. The LIST requires individuals to walk, sprint maximally over 15 m, cruise (fast running) and jog (slower running) back and forth over a 20m distance. In both the studies carried out in Chapters 6 and 7 it was important to gauge the intensity of exercise maintained by the participants. When participants were performing continuous, essentially 'steady state' walking on a treadmill, oxygen uptake measurements using the Douglas bag method provided a valid and reliable means of gauging exercise intensity. In addition other measurements such as heart rate response, subjective feeling of exertion (RPE) and blood lactate concentrations were also measured to allow an assessment of exercise intensity to be made.

During the trials involving intermittent games activity establishing exercise intensity was arguably much more problematic because the intermittent nature of the protocol, involving 40 maximal 15 m sprints and variable speed movement, ensured participants were never in what might be regarded as a 'steady-state'. Also, the nature of the LIST meant that if oxygen uptake measurements were to be made they would need to be done using relatively portable methods. Consequently, it was necessary to use a Douglas bag connected to a rucksack carried on the back of the participant to collect expired air samples. However, the reliability of this system needed to be established. Especially as it was anticipated that the oxygen uptakes would be affected by the intermittent exercise pattern in the LIST trials, and these effects were not well established

Therefore the aim of the investigation outlined below was to establish if measuring oxygen uptake using a Douglas bag connected to a rucksack on the back of a participant was comparable to those made using more established methods.

4.3.2 Methods and Statistical Analysis

Oxygen uptake measurements were made on 14 male participants (age: 22.2 ± 4.4 years; peak $\dot{V}O_2$ 53.0 ± 5.3 ml.kg^{-1}.min^{-1}; height: 182.0 ± 6.3 cm; body mass: 77.0 ± 6.8 kg) on 8 occasions. On each occasion three consecutive 60 s Douglas bag collections were made (see Section 3.4.2). Collections started after participants had been walking uphill on a treadmill for 12 min. Two collections were made into Douglas bags supported on a rack (Douglas bag 1 and 2), the last collection was made into a Douglas bag connected to a rucksack carried on the back of the participant (Rucksack Douglas Bag). The rucksack was carried throughout the three collections, although it was only used for the last sample. The expired air collected in the Douglas bags was analysed using methods described earlier (see Section 3.3.8).

Comparisons were made between the three Douglas bag measurements using a variety of statistical procedures: Pearson Correlation coefficient and the line of identity, one-way ANOVA for correlated samples, mean coefficient of variation, and limits of agreement (Bland & Altman, 1986).

4.3.3 Results

The average oxygen uptakes (mean ± SD) for Douglas bags 1 and 2 and the Rucksack Douglas bag were 2.50 ± 0.36 (n =111), 2.51 ± 0.37 (n =112) and 2.50 ± 0.37 (n =111) l.min^{-1} respectively. Expressed as ml.kg^{-1}.min^{-1} the corresponding values were 32.6 ± 3.6 (n =111), 32.6 ± 3.8 (n =112) and 32.6 ± 3.6 (n =111). No differences in the oxygen uptakes were evident when the values corresponding to Douglas bag 1 and 2 and the Rucksack Douglas bag were analysed using a one-way ANOVA (l.min^{-1}: P = 0.696; ml.kg^{-1}.min^{-1}: P = 0.726). The mean coefficient of variation obtained from analysis of Douglas bags 1 and 2 was 2.2 ± 2.3% (l.min^{-1} and ml.kg^{-1}.min^{-1}:). The comparable value for Douglas bag 2 and the Rucksack Douglas bag was 2.0 ± 1.7%. The mean coefficient of variation from an analysis using all three bags was 2.0 ± 1.7%.

The relationship between the oxygen uptake measurements (l.min^{-1}) made using Douglas bag 1 and 2 and between Douglas bag 2 and the Rucksack Douglas bag are

presented in Figure 4.1. The Pearson correlation coefficients are also shown. Figure 4.2 shows the limits of agreement when the same measurements are compared.

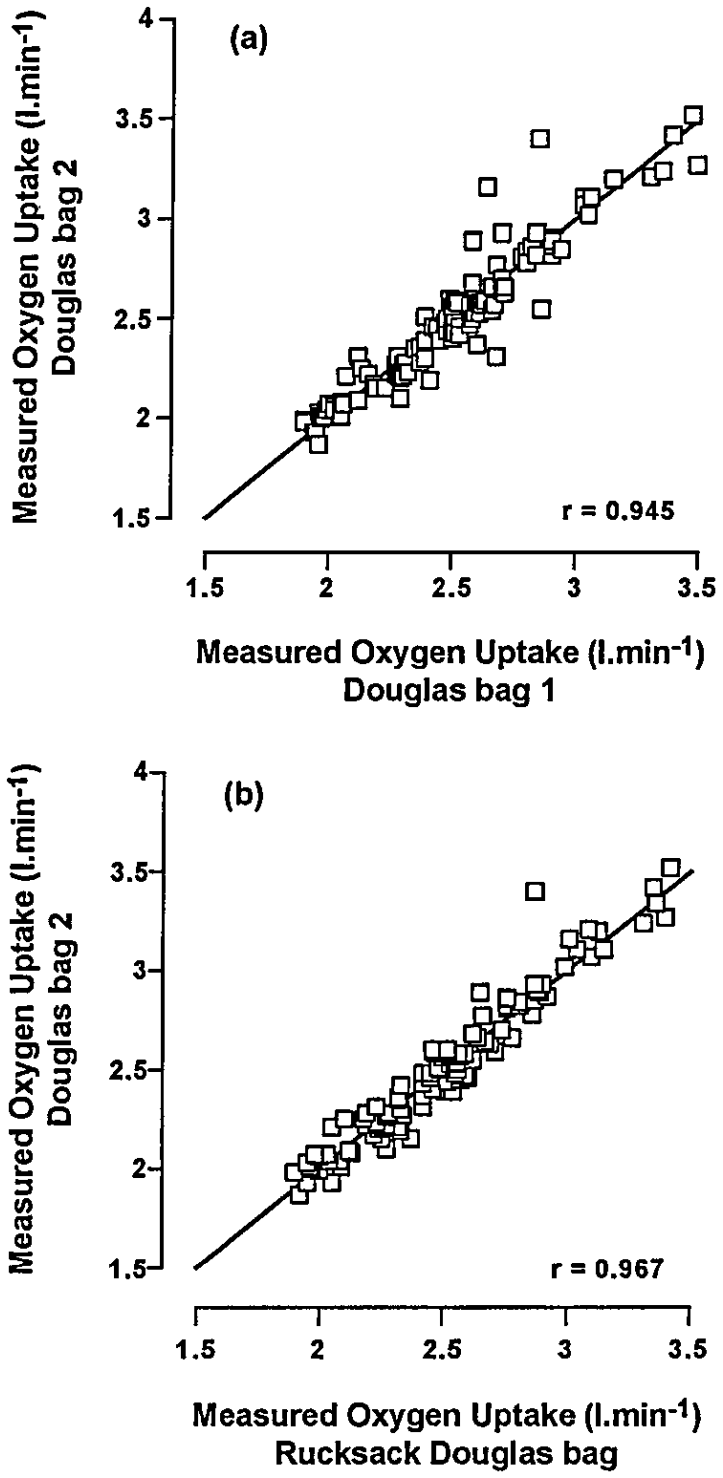

Figure 4.1 The relationship between two consecutive Douglas bag oxygen uptake measurements made using slightly different methods. The relationships shown in (a) and (b) were both significant ($P < 0.001$).

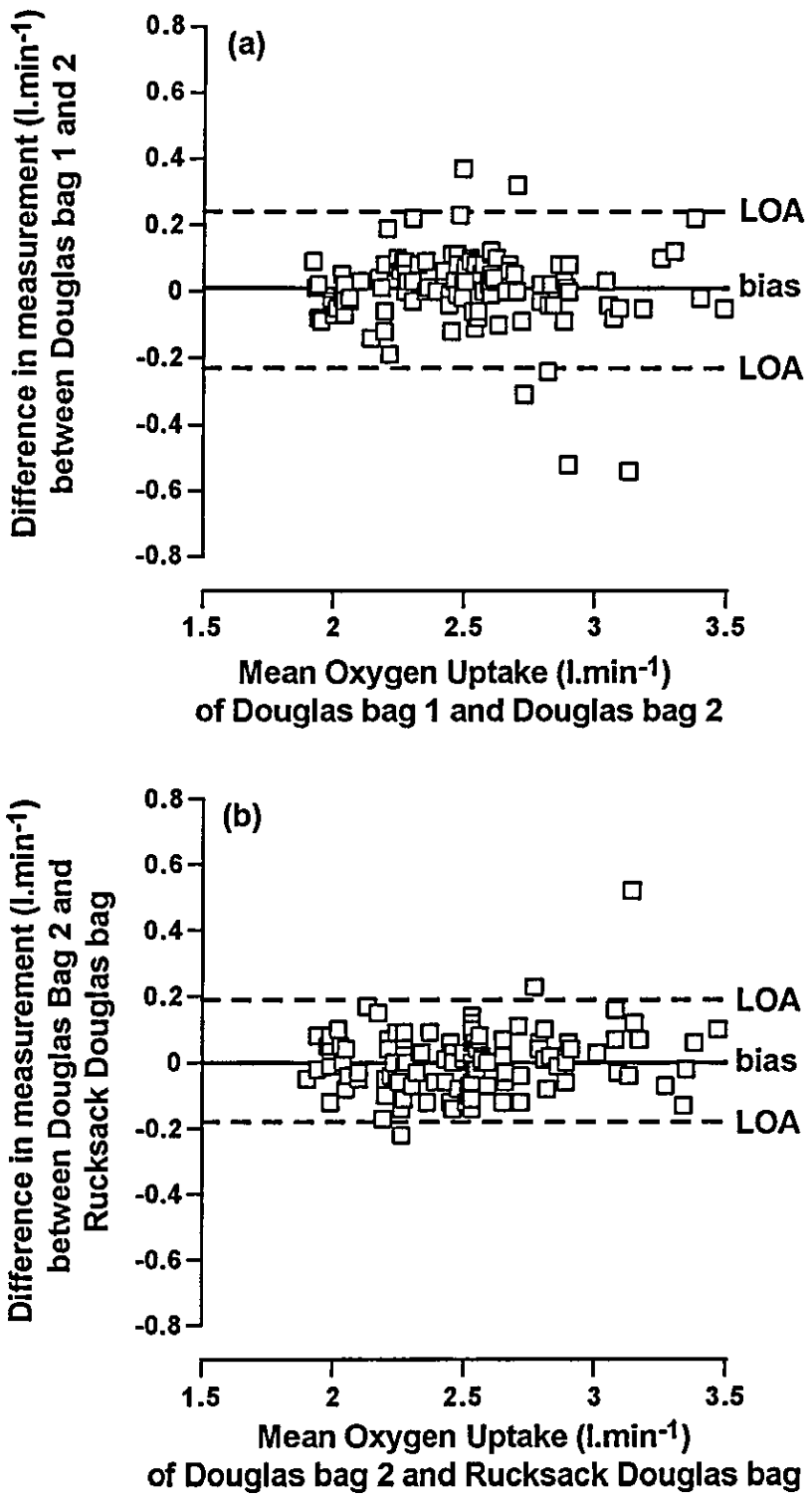

Figure 4.2 The bias and limits of agreement between two consecutive Douglas bag oxygen uptake measurements made using slightly different methods.

The correlation coefficients for the oxygen uptake values expressed in $ml.kg^{-1}.min^{-1}$ were 0.910 ($P < 0.001$; Douglas bag 1 and 2) and 0.945 ($P < 0.001$; Douglas bag 1 and Rucksack Douglas bag). The corresponding bias and limits of agreement were 0.08 and –2.93 – 3.09 ($ml.kg^{-1}.min^{-1}$) and 0.05 and –2.33 – 2.43 ($ml.kg^{-1}.min^{-1}$) for Douglas bag 1 and 2 and Douglas bag 1 and Rucksack Douglas bag respectively.

4 3.4 Discussion

The comparison of oxygen uptake measurements made using Douglas bags 1 and 2 (both secured to a rack) and between 2 and the rucksack Douglas bag suggest the measurements made when the subjects carried the Douglas bag on their backs were as good as those collected more conventionally. This is borne out by the similarity in actual oxygen uptake values, mean coefficient of variations and strong correlations found. The limits of agreement also suggest very similar levels of agreement between the two different methods of oxygen uptake collection.

The mean coefficient of variation of 2.0 ± 1.7%. (mean of 3 measurements) was better than that reported by Carter & Jeukendrup (2002) (3.3 ± 2.5%) at a similar oxygen uptake (~2.5 $l.min^{-1}$), however the mean coefficient of variation was based on an average of 6 measurements in that study as against the 3 used here.

Therefore, based on the findings above, using the Douglas bag mounted on a rucksack would seem to be an adequate method of establishing oxygen uptake during the LIST protocol.

Chapter 5: The Relationship Between Cardiorespiratory Fitness and Blood Lipid-Lipoprotein Profile in Adolescents

5.1 Introduction

Epidemiological studies have identified several important risk factors for CHD. The principle risk factors being: an adverse lipid profile, physical inactivity, hypertension, cigarette smoking, diabetes, obesity and family history of cardiovascular disease (Castelli, 1984). Studies in the US (Berenson *et al.*, 1980) and UK (Boreham *et al.*, 1993) indicate that over 69% of children 12 years of age have at least 1 modifiable risk factor for CHD. In addition there is evidence that a child's risk factor status for CHD tracks into adulthood (Nicklas *et al.*, 2002). A study was performed by McGill and colleagues (2000) to determine whether these risk factors were also associated with early atherosclerosis in young persons by examining arteries and tissue from 3000 autopsied persons age 15-34 y who died of accidental injury, homicide, or suicide. They found that the extent of both fatty streaks and raised lesions (fibrous plaques and other advanced lesions) in the right coronary artery and in the abdominal aorta was associated positively with non-HDL cholesterol concentration, hypertension, impaired glucose tolerance, and obesity and associated negatively with HDL-cholesterol concentration. Although physical activity or regular exercise has been shown to elicit a beneficial effect on blood lipid and lipoprotein profiles in adults (Tran *et al.*, 1983) it is unclear if this is the case in adolescents as the literature is sparse. Therefore the aim of this study was to examine the association between cardiorespiratory fitness (as indicated by peak $\dot{V}O_2$ and percentage peak $\dot{V}O_2$ at a blood lactate concentration of 2.5 mmol.l^{-1}) and blood lipid and lipoproteins in adolescents. It is hypothesised that participants with higher cardiorespiratory levels will have more favourable blood lipid-lipoprotein profiles.

5.2 Methods

5.2.1 Participants

Seventy-seven adolescent males and 62 adolescent females aged between 12 and 16 years of age volunteered to take part in this study, which was approved by the Loughborough University Ethical Advisory Committee. The adolescent participants

who volunteered to take part came from schools in Loughborough, Leicester, Derby and Rugby (32 males and 46 females) and also from various National Governing Body Sports Groups who attended the Nike© Summer Performance Camps at Loughborough University (rugby football league, rugby football union, woman's rugby football union, taekwondo, triathlon and badminton), Nottingham Forest Football Academy and Leicestershire Cricket Academy (45 males and 16 females). Before any testing took place all participants and their primary caregivers had the procedures and risks associated with involvement in the study explained to them (verbally and in writing). Written assent from each participant and consent from their primary caregiver was then obtained. The physical characteristics of the participants are shown in Table 5.1.

Table 5.1 Physical characteristics of participants

	Males $n = 77$	Females $n = 62$
Age (years)	14.5 ± 0.2	14.2 ± 0.2
Height (cm)	165.8 ± 1.4	161.7 ± 1.0 S
Body Mass (kg)	58.0 ± 1.5	53.9 ± 1.3 S
Sum of 7 Skinfolds (mm)	68.4 ± 3.4	98.1 ± 4.4 S
Waist Circumference (cm)	71.5 ± 0 8	66.9 ± 1 0 S
Systolic Blood Pressure (mmHg)	115 ± 1	109 ± 1 S
Diastolic Blood Pressure (mmHg)	65 ± 1	62 ± 1
Tanner Stage Genital/Breast – median (range)	4 (2 – 5)	4 (1 – 5)
Tanner Stage Pubic Hair – median (range)	4 (1 – 5)	4 (2 – 5)
Peak $\dot{V}O_2$ (ml.kg.min^{-1})	55.8 ± 0.8	44.3 ± 0.6 S
% Peak $\dot{V}O_2$ at a BLa of 2.5 mmol.l^{-1} Males $n = 33$ and Females $n = 18$	68.4 ± 2.8	73.8 ± 1.7

Values are mean ± SEM

S main effect sex (height, $P = 0$ 019; body mass, $P = 0.048$; sum of 7 skinfolds, $P < 0.001$; waist circumference, $P < 0.001$; systolic blood pressure, $P = 0.001$; peak $\dot{V}O_2$, $P < 0.001$).
*Abbreviation:*Bla = blood lactate concentration

5.2.2 Preliminary Measurements

Height and body mass were measured using standard methods (see Section 3.3.1). Skinfold thicknesses were measured at seven sites (triceps, biceps, subscapula, supra-iliac, abdominal, anterior thigh and medial calf) using callipers, according to the protocol described by Cameron (1984) (see Section 3.3.2). Circumferences were measured at five sites (upper-arm, waist, hip, thigh and calf) (see Section 3 3 2). Each participant was asked to make a self-assessment of sexual maturity using a five-point scale described by Tanner (1962) to assess the development of breasts in girls, genitalia in boys and pubic hair in both sexes (see Section 3 3.4). Blood pressure was measured by auscultation using a mercury sphygmomanometer (see Section 3.3.3).

5.2 3 Lipid–Lipoprotein Profile

Following an overnight fast, capillary blood samples were collected (see Section 3.5) for the immediate determination of total cholesterol, HDL cholesterol and TAG concentration using a dry chemistry method (Reflotron, Boehringer Mannheim, Germany) (see Section 3 6.5).

5.2.4 Measuring Cardiorespiratory Fitness

Each participant performed a continuous, incremental treadmill test which comprised of 4 min stages of increasing exercise intensity to determine their sub maximal oxygen uptake and blood-lactate (in a subgroup of 33 males and 18 females) responses (see Section 3.3 8). Peak oxygen uptake was determined using a continuous, progressive uphill run to volitional fatigue (see Section 3.3.9).

5.2.5 Statistical Analysis

Multiple-regression and Person product moment correlations were used to analyse the data. A number of diagnostic checks, including checks of the residuals (for example checks for normality) were under taken to assess the stability/integrity of the regression analysis. The HDL cholesterol data were log transformed (lnHDL), as they were not found to be normally distributed. This was done to stabilise the variances and improve symmetry and normality. Data was analysed using Minitab.

5.3 Results

The blood lipid–lipoprotein profile of the participants is shown in Table 5.2. Although TAG concentration was measured the data is not presented as only 10 males and 12 females had concentrations higher than 0.80 mmol.l^{-1}, which is the lower limit of detection of the Reflotron analyser. The male participants were taller, heavier and leaner than the female participants. They also had larger waist circumferences, higher systolic blood pressure, higher peak $\dot{V}O_2$ and lower total cholesterol concentrations.

Table 5.2 Lipid-lipoprotein concentrations

	Males $n = 77$	Females $n = 62$
Total Cholesterol (mmol.l^{-1})	3.44 ± 0.06	3.70 ± 0.08^{S}
HDL Cholesterol (mmol.l^{-1})	1.10 ± 0.04	1.19 ± 0.03
Total Cholesterol : HDL Cholesterol	3.17 ± 0.12	3.15 ± 0.08

Values are mean ± SEM

^{S} main effect sex, $P = 0\ 009$

Using correlation analysis, a relationship was found between peak $\dot{V}O_2$ and lnHDL cholesterol and the total cholesterol/HDL cholesterol ratio ($r = 0.247$, $P = 0.042$; $r = -0.355$, $P = 0.003$) and between percentage peak $\dot{V}O_2$ at a blood lactate concentration of 2.5 mmol.l^{-1} and the total cholesterol/HDL cholesterol ratio ($r = -0.360$, $P = 0.047$) in males and between peak $\dot{V}O_2$ and the total cholesterol/HDL cholesterol ratio ($r = -0.315$, $P = 0.040$) in females. When both sexes were analysed together a relationship was found between peak $\dot{V}O_2$ and total cholesterol and the total cholesterol/HDL cholesterol ratio ($r = -0.218$, $P = 0.021$; $r = -0.200$, $P = 0.037$ respectively) and between percentage peak $\dot{V}O_2$ at a blood lactate concentration of 2.5 mmol.l^{-1} and the total cholesterol/HDL cholesterol ratio ($r = -0.300$, $P = 0.041$). Using multiple regression analysis, peak VO_2 remained an important predictor of HDL cholesterol and the total cholesterol/HDL cholesterol ratio, as were sex and waist circumference [lnHDL cholesterol = 0.335 + 0.137 Sex01 + 0.007798 Peak $\dot{V}O_2$ (ml.kg.min^{-1}) – 0.0100 Waist circumference (cm); Total cholesterol / HDL cholesterol = 3.79 – 0.533 Sex01 – 0.0447 Peak $\dot{V}O_2$ (ml kg.min^{-1}) + 0.0279 Waist circumference (cm)]. These three predictor variables explained 17.1% of the variation in lnHDL cholesterol

concentrations and 12.6% of the variation in the total cholesterol/HDL cholesterol ratio. When it was attempted to enter group (school or camps), age, weight, height, systolic or diastolic blood pressure, any of the maturity ratings, skinfold values or other circumference values, none were required (not significant) over an above the three predictors (sex, peak $\dot{V}O_2$ and waist circumference). Skinfold values were entered as individual values and in a number of combinations (sum of 7 measurements, sum of 4 measurements (biceps, triceps, subscapular and supra-iliac), peripheral (triceps, biceps, thigh and calf) and central (supscapular, supra-iliac, abdominal). All the individual circumference measures were also entered, as well as waist to hip ratio.

5.4 Discussion

The main finding of this study was that peak $\dot{V}O_2$, sex and waist circumference were found to be important predictors of lnHDL cholesterol and the total cholesterol/HDL cholesterol ratio in a group of adolescents. These three predictor variables explained 17.1% of the variation in lnHDL cholesterol concentrations and 12.6% of the variation in the total cholesterol/HDL cholesterol ratio. Significant correlations were also found between peak $\dot{V}O_2$ and the total cholesterol/HDL cholesterol ratio in both males and females (r = 0.247, P = 0.012; r = 0 315, P = 0.047 respectively). In addition a positive relationship was found between peak $\dot{V}O_2$ and lnHDL cholesterol, and a negative relationship between % peak $\dot{V}O_2$ at 2.5 mmol.l^{-1} blood lactate concentration and the total cholesterol/HDL cholesterol ratio in males only (r = 0 247, P = 0.012; r = -0.360, P = 0 047 respectively). When both sexes were analysed together negative relationships were found between peak $\dot{V}O_2$ and total cholesterol and the total cholesterol/HDL cholesterol ratio (r = -0.218, P = 0.021; r = -0.200, P = 0.037 respectively) and between percentage peak $\dot{V}O_2$ at a blood lactate concentration of 2.5 mmol.l^{-1} and the total cholesterol/HDL cholesterol ratio (r = -0.300, P = 0.041).

The peak $\dot{V}O_2$ values of the participants in this study were slightly higher than those reported for other British children of the same age (mean values 56 and 44 ml.kg^{-1}.min^{-1} in this study compared with 48 and 41 ml.kg^{-1}.min^{-1}, males and females respectively) (Armstrong *et al.*, 1991). Body fatness as indicated by the sum of triceps and subscapular were similar (mean values 17.1 and 24.6 mm in this study

compared with 20 0 and 25.6 mm males and females respectively). Both total cholesterol and HDL cholesterol concentrations were lower than those reported for British children in other studies (Orchard *et al.*, 1980; Armstrong *et al*, 1992). This is probably due to differences in the methods used to measure blood lipid and lipoprotein concentrations (see Section 3.10 4).

The relationships found between fitness variables and blood lipid-lipoprotein concentrations seen in this study could possible be explained by the slightly higher peak $\dot{V}O_2$ than those reported in other studies of British children of the same age. In addition unlike a number of other studies this study had a large number of participants who were involved in various different sports at a high level (international, regional and county). As it can be argued that peak $\dot{V}O_2$ is largely genetically determined a measure of cardiovascular fitness that would give an indication of how well trained the participants in this study were, % peak $\dot{V}O_2$ at 2.5 mmol.l^{-1} blood lactate concentration was also used. To the knowledge of the author this is the only study to examine the relationship between blood lipid-lipoprotein profile and this measure of cardiovascular fitness. A negative relationship between percentage peak $\dot{V}O_2$ at a blood lactate concentration of 2.5 mmol.l^{-1} and the total cholesterol/HDL cholesterol ratio (r = -0.300, P = 0.041) was seen when both sexes were analysed together and also in males (r = -0.360, P = 0.047). It is unclear why this relationship was not seen when the females were analysed separately. Percentage peak $\dot{V}O_2$ at 2.5 mmol.l^{-1} blood lactate concentration was not found to be a predictor of any of the blood lipid-lipoprotein measures. This could be due to the low number of participants in this particular analysis which might have resulted in a lack of statistical power.

It is interesting that waist circumference has been found to be a predictor of lipid-lipoprotein profile in adolescents as it has been suggested that waist circumference can be used alone as an anthropometric indicator of health risk in adults (Executive Summary, 1998) since a positive correlation has been found between waist circumference and abdominal fat content. The presence of excess fat in the abdomen out of proportion to total body fat is an independent predictor of risk factors and morbidity from CHD. The fact that the male participants in this study had higher circumference measurements than the females but lower total cholesterol concentrations can be explained by the fact that the male participants also had lower

skinfold values. This indicates that their higher circumference measurements were not due to higher levels of body fat.

It should be noted that there are a number of potential problems associated with performing this type of analysis in a group of young people. The incidence of morbidity and mortality related to CHD is extremely low in a paediatric population (usually only seen in young people with genetic conditions such as familial hypercholesterolaemia, if not adequately treated). Therefore studies investigating the relationship between physical activity and cardiovascular health in children and adolescents are mostly limited to cardiovascular risk factors. It has been suggested that, it is unlikely that some markers of health outcomes of inactivity will be sufficiently clear until early or late adulthood, even though inactivity in youth may be a precursor to such effects (Biddle *et al.*, 2004). This could explain why as in this study only weak correlations and associations between physical activity/ cardiorespiratory fitness and blood lipid-lipoprofile (if any) have been seen and the relatively low percentage of variance in the blood lipid values explained by the three predictors (peak $\dot{V}O_2$, sex and waist circumference). Furthermore, it is possible that most children and adolescents have normal lipid/lipoprotein profiles that are not amenable to change by exercise (Després *et al.*, 1990). Therefore it may be more appropriate to focus on groups of young people who already exhibit signs of unfavourable CHD risk profiles. It has also been noted that this type of analysis cannot identify threshold values above which beneficial effects will occur (Twisk, 2001). Therefore it does not provide any evidence on which physical activity guidelines can be based.

The use of volunteers is a limitation to the applicability of the results of this study. The fact that the adolescents desired to participate in this study despite the discomfort of a maximal exercise test may indicate something about their nature. It is very unlikely that any of the participants in this study were truly sedentary. A narrow range of peak $\dot{V}O_2$ values could disguise any relationship which would be found in the larger population.

The participants in this study also spanned a wide maturity range. Children and adolescents are not as stable physiologically as adults. This is as a result of numerous

changes they are undergoing as a result of the growth process. It has been established that HDL cholesterol decreases during puberty in males (Orchard *et al*, 1980). Moreover this decrease was not found to be attenuated by training, even in highly endurance trained adolescents (Eisenmann *et al.*, 2001). This suggests that the influence of training is not strong enough to overcome the normal processes of growth. This is a factor, which should be considered when examining the results of this study.

When we performed some preliminary analysis with a lower number of participants (66 males and 26 females, who have also been included in this study) (Barrett *et al*, 2004) height was found to be a predictor of HDL cholesterol and the total cholesterol/HDL cholesterol ratio in both males and females. In addition a correlation between HDL cholesterol and peak $\dot{V}O_2$ was found in the female participants (r = 0.573, P = 0.005). This suggests that sample size may affect the findings of studies examining the effect of physical activity and/or cardiovascular fitness.

In conclusion the results of this study indicate that for adolescents, peak $\dot{V}O_2$, sex and waist circumference may be important determinants of blood lipid-lipoprotein profile. These results support the view that it is important for young people to lead an active lifestyle as this could possibly increase their levels of cardiovascular fitness.

Chapter 6: Effects of Intermittent Games Activity on Postprandial Lipaemia in Adults

6.1 Introduction

In Chapter 5 the effect of exercise on blood lipoprotein concentration in the fasted state was examined. Although the concentrations of lipoproteins in the fasted state can be important predictors of cardiovascular risk they provide little information about the mechanisms by which exercise influences the lipoprotein profile. In addition, while many of the postprandial changes in lipid metabolism are transient in nature, the customary intake of food in Western societies in three meals spread through the day means that the metabolic disturbances associated with one meal are unlikely to have subsided before another meal is ingested. As a result of this people can spend up to two thirds of the day in the postprandial state and therefore from a metabolic viewpoint this could be thought of as the "normal" physiological state (Gill & Hardman, 2003). Exaggerated postprandial lipaemia indicates poor TAG metabolic capacity (Miesenböck & Patsch, 1992) and has been implicated in the development of atherosclerosis. Therefore interventions to reduce postprandial lipaemia could potentially slow atherogenic progression. Several studies have shown that a single session of exercise can reduce postprandial lipaemia (Aldred *et al.*, 1994; Tsetsonis & Hardman, 1996b; Tsetsonis & Hardman, 1996a; Tsetsonis *et al*, 1997; Malkova *et al*, 1998; Zhang *et al.*, 1998). It appears that exercise induced reductions in postprandial lipaemia are linked to the energy expenditure of an exercise session. Increasing the energy expenditure of an exercise session by increasing either the duration (Annuzzi *et al.*, 1987) or exercise intensity (Tsetsonis & Hardman, 1996b) has been found to result in a greater reduction in lipaemic response. Also similar energy expenditures at different exercise intensities have been found to elicit similar attenuations in postprandial lipaemia (Tsetsonis & Hardman, 1996a). To date only the effects of continuous modes of exercise such as treadmill walking or running have been determined. As many people take part in intermittent games type activity such as hockey, rugby or soccer it is important to investigate what effect this mode of exercise has on postprandial lipaemia. In addition, as the aim of the next study was to examine the effects of exercise on adolescents it was important to determine if this mode of exercise could reduce postprandial lipaemia in adults before trying to establish if this was the case in adolescents. Therefore the present study sought to examine the

influence of intermittent games activity on postprandial lipaemia in a group of young adult males and to test the hypothesis that if the energy expenditure was sufficient a single session of this type of exercise would reduce postprandial lipaemia

6.2 Methods

6.2.1 Participants

Twelve, healthy, recreationally active males volunteered to take part in this study, which was approved by the Loughborough University Ethical Advisory Committee. After fully explaining the procedures and risks involved, written consent to take part was obtained from each participant before any testing took place. Participants were only recruited if they met the following criteria: were non-smoking, were free of known cardiovascular disease or abnormalities, were not taking any mediation known to influence lipid or carbohydrate metabolism, had resting arterial blood pressure < 160/95 mmHg, and had a BMI < 35 kg/m^2. The physical characteristics of the participants are shown in Table 6.1.

Table 6.1 Physical characteristics of participants

Age (years)	21.1 ± 0.4
Height (cm)	183.0 ± 1 8
Body Mass (kg)	76.9 ± 1.8
BMI	23.0 ± 0.5
Sum of Skinfolds (mm)	37.9 ± 3.6
Peak $\dot{V}O_2$ ($ml.kg^{-1}.min^{-1}$)- treadmill	53.0 ± 1.5
Peak $\dot{V}O_2$ ($ml.kg^{-1}.min^{-1}$)- predicted (MSST)	52.7 ± 1.5

Values are mean ± SEM, $n = 12$
Abbreviation MSST = Multistage Shuttle Run Test

6.2.2 Anthropometry

Height and body mass were measured using standard methods (see Section 3.3.1). Skinfold thicknesses were measured at four sites (triceps, biceps, subscapula and supra-iliac) using callipers, according to the protocol described by Cameron, (1984) (see Section 3.3.2). BMI was calculated as the body mass in kilograms divided by the square of the height in metres. Waist circumference was taken as the minimum diameter measured between the iliac crests and lower ribs. Hip circumference was

measured at the level of greatest protrusion of the buttocks and taken as the maximum diameter (see Section 3.3.2).

6.2.3 Preliminary Tests

A series of preliminary tests were completed prior to the main trials. Blood pressure was measured by auscultation using a mercury sphygmomanometer (see Section 3.3.3). Peak oxygen uptake was measured directly using an incremental, uphill treadmill walking test to exhaustion using the Douglas bag method. This test was also used to establish the relationship between oxygen uptake, speed and gradient in order to set the gradient necessary to elicit 60% of their peak $\dot{V}O_2$ at their self-selected speed for the Walk main trial (see Section 3.3.9). In addition peak $\dot{V}O_2$ was estimated using a progressive multistage shuttle run test (MSST) (Ramsbottom *et al.*, 1988) (see Section 3 3.5). Individuals performed two familiarisation sessions. This was in order that they became accustomed with the Walk and the Loughborough Intermittent Shuttle Test (LIST) protocols and to set and check the exercise intensity for the main trials. The LIST was used in this study to simulate games activity and was a modified version of the test reported by Nicholas and colleagues (2000). An attempt was made to set the exercise intensity for both exercise modes at an average of 60% $\dot{V}O_2$ peak (determined using oxygen uptake values measured during the rest periods) over each block. Participants were required to perform 4 blocks of uphill walking or the LIST on two separate occasions. This replicated the amount of exercise performed during the main trials. The LIST protocol required participants to repeat a pattern of exercise, which consisted of a walk, sprint, run ("cruise" at 70% of $\dot{V}O_2$ peak) and jog (at 40% of $\dot{V}O_2$ peak) over a marked 20 m distance in a sports hall. This pattern was repeated 10 times forming one "block" of exercise (see Section 3.4.2). The running speeds were lowered until the intensity achieved was as close as possible to 60% of $\dot{V}O_2$ peak, without the running speeds becoming so slow that the participants had to walk.

6.2.4 Study Protocol

Each participant took part in three main trials: a Rest trial, a Walk exercise trial and a LIST exercise trial a minimum of 6 days apart, in a balanced cross-over design. During the Rest trial participants refrained from exercise on day one of the

experiment and then performed an oral fat tolerance test on the morning of day two. During the exercise trials on the afternoon of day one, participants performed a 15 min warm-up on a treadmill consisting of 7 min of walking at a 1% gradient, at the speed maintained during the peak $\dot{V}O_2$ test, 5 min of stretching and 3 min of walking at a speed and gradient selected to elicit 60% of peak VO_2. Participants then completed either four blocks of treadmill walking or four blocks of the LIST, with 3 min rest between each block. The next morning (day two) participants attended the laboratory for a fat tolerance test. Exercise was completed at the same time of day (between 3:30 and 5 pm) in both exercise trials so that the time interval between the end of the exercise sessions and beginning of the fat tolerance tests was similar in both exercise trials ($\approx$16 h)

Participants were asked to refrain from physical activity for the 2 days before the commencement of each trial and also to weigh and record their food and drink intake during the two day period before the oral fat tolerance test of their first trial They were asked to replicate this diet for all subsequent trials. Participants were also asked not to consume alcohol during these periods.

On day two of each trial participants underwent an oral fat tolerance test (see Section 3.4.3). Blood was sampled (fasting, 0.5, 0.75, 1, 2, 3, 4, 5 and 6 h, see Section 3.5) and stored for later analysis (see Section 3.6) Expired air collections were also made (fasting, 2.5 and 5.5 hours, see Section 3.4.5). The test meal employed was a high fat, mixed meal comprising of cereal, fruit, nuts, chocolate and whipping cream, with the whipping cream being the main contributor to the fat load. The meal provided 1.25 g fat, 1.07 g carbohydrate and 0 20 g protein per kg body mass (see Section 3.4.4).

6.2.5 Statistical Analysis

The 6 h area under the plasma concentration versus time curves for TAG, NEFA, glucose and insulin were calculated using the trapezium rule. The incremental area under the curve for TAG was calculated using the same method after correcting for baseline concentrations. Fasting concentrations for total cholesterol, HDL cholesterol, TAG, NEFA, glucose and insulin and the area under the curve values for TAG, NEFA, glucose and insulin were compared between trials using one-way ANOVA for correlated means, using a Bonferroni adjustment to establish were

differences lay. Two-way ANOVA (trial - time) was used to determine differences between trials and over time for fasting and postprandial plasma concentrations of TAG, NEFA, glucose and insulin as well as for RER, energy expenditure, carbohydrate and fat oxidation and to compare lactate, heart rate, % HRmax, % peak $\dot{V}O_2$, RPE, and block time from the exercise trials. A Bonferroni adjustment was used to establish were differences lay. Statistical significance was accepted at the $P < 0.05$ level. Data were analysed using the Statistical Package for Social Science (SPSS) software version 11.0 for windows (SPSS Inc, Chicago, IL). Data are presented as means ± standard error of the mean (SEM) and are based on a population of twelve unless otherwise stated.

6.3 Results

6 3.1 Reponses During Exercise Trials

The averages shown in Table 6.2 are based on the mean of measurements made over the 4 blocks of exercise.

Table 6.2 Lactate, heart rate, percentage of maximum heart rate (% HR max), percentage of peak oxygen uptake (% peak $\dot{V}O_2$), rate of perceived exertion (RPE) and time to complete each exercise block (Block time) during the Walk and LIST trials

	Walk	LIST
Lactate ($mmol.l^{-1}$)	2.4 ± 0.3	4.3 ± 0.6 ^{T}
Heart rate ($beats.min^{-1}$)	146 ± 3	160 ± 3 ^{T,i}
% HR max	77.0 ± 2.1	85.1 ± 1.8 ^{T,i}
% Peak $\dot{V}O_2$ Treadmill	61.6 ± 1.0	71.8 ± 1.6 ^{T}
RPE median (range)	12 (9 – 15)	12 (8 – 16) ^{t,i}
Block time (min:sec)	15:00 ± 00:00	16:15 ± 00:14 ^{T}

Values are mean ± SEM, $n = 12$

^{T} main effect trial (lactate, $P = 0.002$; HR, $P < 0.001$; % HR max, $P < 0.001$; % peak $\dot{V}O_2$, $P < 0.001$; Block time, $P = 0.001$). ^{t} main effect time (RPE, $P = 0.001$). ^{1} interaction trial – time (HR, $P = 0.028$; % HR max, $P = 0.028$; RPE, $P = 0.012$)

Lactate, heart rate, % HR max and % peak $\dot{V}O_2$ were all found to be lower during the Walk than in the LIST trial (lactate main effect trial, $P = 0.002$; main effect time, n.s.;

interaction trial - time, n.s.; HR main effect trial, $P < 0.000$; main effect time, n.s.; interaction trial - time, $P = 0.028$; % HR max main effect trial, $P < 0.000$; main effect time, n s ; interaction trial - time, $P = 0.028$; % peak $\dot{V}O_2$ main effect trial, $P < 0.001$; main effect time, n.s.; interaction trial - time, n.s.). Also the time to complete each exercise block was on average 1 min 15 s shorter during the Walk than the LIST trial (main effect trial, $P = 0\ 001$; main effect time, n.s.; interaction trial - time, n.s.). The ratings of perceived exertion were not found to differ between trials (main effect trial, n.s.; main effect time, $P = 0\ 001$; interaction trial - time, $P = 0.012$). The mean gross energy expenditure for the Walk was 3 1 ± 0 2 MJ or 40.2 ± 1.5 $kJ.kg^{-1}$ body mass. The energy expenditure for the LIST was not calculated due to the methodological difficulties in calculating energy expenditure in non steady state exercise when the RER is above unity.

6.3.2 Plasma Concentrations in the Fasted State

Plasma concentrations in the fasted state are shown in Table 6.3. There were no differences in fasting plasma concentrations of total cholesterol, HDL cholesterol, NEFA or glucose between trials. Fasting plasma TAG and insulin concentrations were lower in the LIST trial than the Rest trial (main effect trial, $P = 0.022$ and $P = 0.032$ respectively).

Table 6.3 Fasting plasma concentrations of total cholesterol, HDL cholesterol, TAG, NEFA, glucose and insulin in the Rest, Walk and LIST trials

	Rest	Walk	LIST
Total Cholesterol ($mmol.l^{-1}$)	3.83 ± 0.13	3.74 ± 0.16	3.88 ± 0.17
HDL-Cholesterol ($mmol.l^{-1}$)	1.27 ± 0 05	1.26 ± 0.06	1.24 ± 0 06
TAG ($mmol.l^{-1}$)	0.95 ± 0.06	0.80 ± 0.07	0.72 ± 0 07 T
NEFA ($mmol.l^{-1}$)	0.36 ± 0.05	0.51 ± 0.07	0.45 ± 0.05
Glucose ($mmol.l^{-1}$)	4.90 ± 0.06	5.06 ± 0.16	5.03 ± 0.15
Insulin ($\mu U.ml^{-1}$)	16.03 ± 1.00	16.49 ± 1.29	12.93 ± 1.14 T

Values are mean ± SEM, $n = 12$

T Significantly different from rest trial (main effect trial, TAG, $P = 0.022$; insulin, $P = 0\ 032$)

6.3.3 Estimated Change in Plasma Volume

Changes in plasma volume over the period of observation were small (Rest, 2.2 ± 1.0 %; Walk, -0.6 ± 1.6 %; LIST, 1.6 ± 1.6 %) and did not differ between trials, therefore concentrations were not corrected in this respect.

6.3.4 Postprandial Plasma Responses to the Fat Tolerance Tests

Plasma TAG response to the test meals and the total and incremental areas under the TAG concentration versus time curve are shown in Figure 6.1. Postprandial TAG concentrations were found to be lower during the LIST than the Rest trial (main effect trial, $P = 0.001$; main effect time, $P < 0.001$; interaction trial - time, n s.). Concentrations were also lower in the Walk trial compared to the Rest trial but the difference was not found to be significant (main effect trial, $P = 0.053$). In addition the total area under the plasma TAG versus time curve was lower in the LIST than the Rest trial (reduced by 25%, $P = 0.001$). While the total area under the TAG curve was reduced by 19% when the Walk was compared with the Rest trial this change was not statistically significant ($P = 0.064$). Although a main effect of trial was found in the incremental areas under the curves for TAG (P = 0.038), the location of the difference/s could not be found using a Bonferroni adjustment, however it is reasonable to assume that there is a difference between the highest and the lowest values, that is between the Rest and the LIST trials.

The plasma NEFA, glucose and insulin responses to the test meals are shown in Figure 6.2. The total areas under the concentration versus time curves for these parameters are given in Table 6 4. No differences were found in postprandial glucose concentrations between trials (main effect trial, n s.; main effect time, $P = 0\ 003$; interaction trial - time, n.s.). A difference was found however, in postprandial NEFA and insulin concentrations between trials (NEFA main effect trial, $P = 0.042$; main effect time, $P < 0.001$; interaction trial - time, $P = 0.088$; Insulin main effect trial, $P = 0.011$; main effect time, $P < 0.001$; interaction trial - time, $P = 0.164$). Postprandial NEFA concentrations were found to be higher during the LIST than the Rest trial (main effect trial, $P = 0.027$). Postprandial insulin concentrations were found to be lower in the LIST than the Rest (main effect trial, $P = 0.002$) and Walk trials (main effect trial, $P = 0.031$). No statistical differences were found in the total areas under the concentration versus time curves for glucose between trials

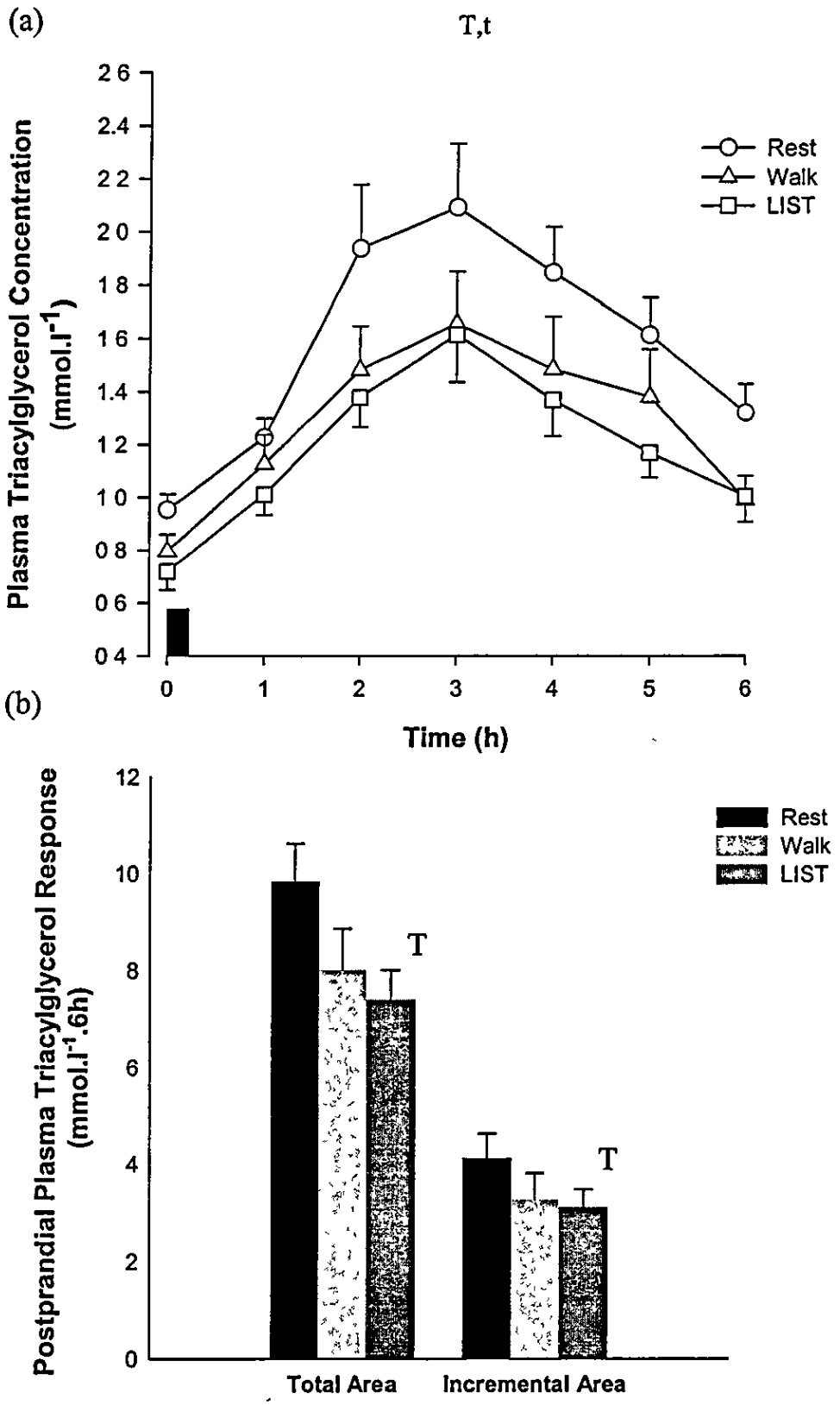

Figure 6.1 Fasting and postprandial TAG concentrations for Rest, Walk and LIST trials (a), black rectangle indicates consumption of test meal (^{T} main effect trial, $P = 0.001$; ^{t} main effect time, $P < 0.001$; interaction trial - time, n s.). Total and incremental areas under the TAG concentration versus time curve (b). ^{T} Significantly different from Rest (total area, $P = 0.001$; incremental area $P = 0\ 038$ using 1 – way ANOVA) Values are mean ± SEM, $n = 12$

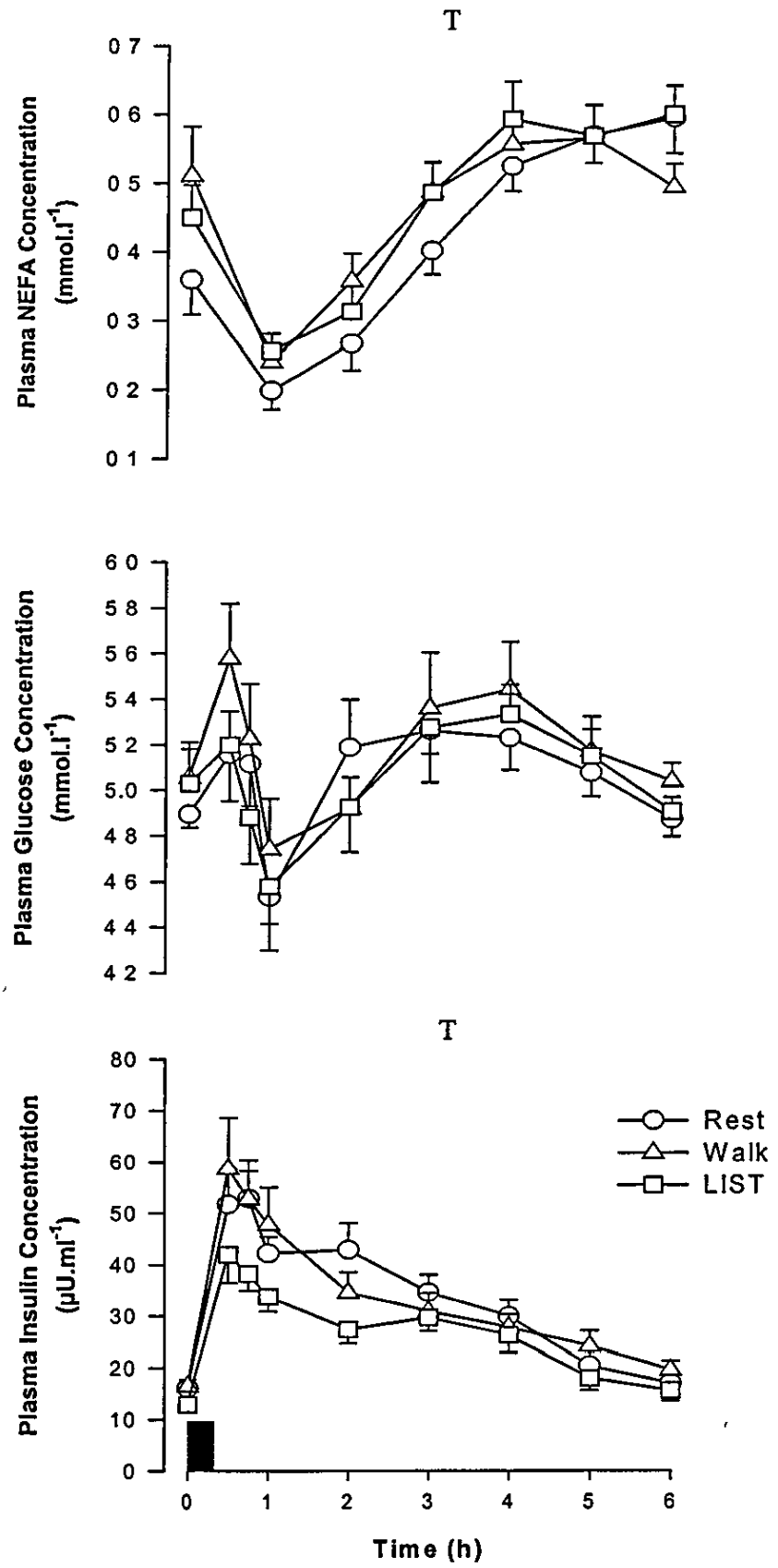

Figure 6.2 Fasting and postprandial insulin, glucose and non-esterified fatty acid (NEFA) concentrations for the Rest, Walk and LIST trials, black rectangle indicates consumption of test meal, ^{T}significant differences were found in insulin between Rest and LIST (main effect trial, P = 0 002) and LIST and Walk (main effect trial, P = 0.031) and in NEFA between Rest and LIST (main effect trial, P = 0.027). Values are mean ± SEM, *n* = 12

Although a main effect of trial was found for NEFA (P = 0.032), the location of the difference/s could not be found using a Bonferroni adjustment, however it is reasonable to assume that there is a difference between the highest and the lowest values, that is between the Rest and the LIST trials. The total areas under the concentration versus time curves for insulin were statistically lower in the LIST than the Rest ($P = 0.004$) trial. While the total areas were also lower in the LIST than the WALK trials, the difference was not significant ($P = 0.063$).

Table 6.4 Six hour area under the plasma concentration versus time curves for NEFA, glucose and insulin in the Rest, Walk and LIST trials

	Rest	Walk	LIST
NEFA (mmol.l^{-1}.6 h)	2.44 ± 0.15	2.71 ± 0.17	2.74 ± 0.18 ^{T}
Glucose (mmol.l^{-1}.6 h)	30.46 ± 0.70	31.04 ± 0.76	30.43 ± 0.47
Insulin (μU.ml^{-1}.6 h)	199.3 ± 14.7	196.4 ± 17.3	158.9 ± 9.4 ^{T}

Values are mean ± SEM, $n = 12$

^{T} Significantly different from Rest (main effect trial, NEFA, $P = 0.032$; Insulin, $P = 0.004$)

6.3 5 Substrate Utilization and Energy Expenditure

Fasting and postprandial values for energy expenditure, respiratory exchange ratio (RER), fat oxidation and carbohydrate oxidation are shown in Table 5.5. Carbohydrate oxidation was higher in the Rest than in the LIST trial (main effect trial, $P = 0.007$). A main effect of time was also found in carbohydrate oxidation and RER ($P = 0.013$ and $P = 0.049$ respectively). Carbohydrate oxidation was found to be higher at 2.5 h than fasting (main effect time, $P = 0.04$) and than at 5.5 h (main effect time, $P = 0.008$). Respiratory exchange values were found to be higher at 2.5 h than fasting (main effect time, $P = 0.023$).

Table 6.5 Substrate utilization and energy expenditure in the fasted and postprandial states for the LIST group

	Fasting	2.5 h	5.5 h	Mean
Energy Expenditure ($kJ.h^{-1}$)				
Rest	313 ± 10	318 ± 14	286 ± 8	306 ± 11
Walk	326 ± 12	338 ± 12	317 ± 8	327 ± 11
LIST	296 ± 7	339 ± 16	310 ± 10	315 ± 11
RER				
Rest	0 78 ± 0.01	0.80 ± 0.01	0.76 ± 0.02	0.78 ± 0.01
Walk	0.77 ± 0.01	0.77 ± 0.01	0.75 ± 0.01	0.76 ± 0.01
LIST	0.75 ± 0.01	0.75 ± 0.01 t	0.73 ± 0.01	0.74 ± 0.01
Fat Oxidation ($g.h^{-1}$)				
Rest	5.96 ± 0.45	5.41 ± 0.50	5.72 ± 0.50	5.70 ± 0.48
Walk	6.40 ± 0.31	6.51 ± 0.27	6.71 ± 0.39	6.54 ± 0.33
LIST	6.24 ± 0.31	7.07 ± 0.43	6.98 ± 0.32	6.77 ± 0.35
Carbohydrate Oxidation ($g.h^{-1}$)				
Rest	4.84 ± 0.74	6.57 ± 0.94	4.68 ± 0.76	5.36 ± 0.81
Walk	4.58 ± 0.63	5.10 ± 0.77	3.22 ± 0.74	4 30 ± 0.71
LIST	3.07 ± 0.74	3.68 ± 0.64 t	2.04 ± 0.43 t	2.93 ± 0 60 T

Values are mean ± SEM, $n = 10$

T main effect trial, significant difference between Rest and LIST ($P = 0.007$), t main effect time, significant difference between RER at fasting and 2.5 hours ($P = 0.023$), between carbohydrate oxidation at fasting and 2.5 hours ($P = 0\ 044$) and between 2 5 hours and 5.5 hours ($P = 0.008$)

6.4 Discussion

The main finding in the present study was that a single session of intermittent games activity performed 16 h prior to an oral fat tolerance test reduced postprandial lipaemia in a group of young adult males. While TAG concentrations were also lower in the Walk trial compared to the Rest trial the difference was not found to be significant at the $P < 0.05$ level (main effect trial, $P = 0.053$). The total area under the plasma TAG versus time curve was reduced by 25% and 19% in the LIST and Walk exercise trials respectively when compared to the Rest trial. However, the reduction seen in the Walk trial was not statistically significant (main effect trial, $P = 0.064$).

Although the mediating factors are not completely understood, it seems likely that exercise reduces postprandial TAG concentration by two complementary

mechanisms. One is thought to involve increased LPL activity in the skeletal muscle (Oscai *et al.*, 1990), which may enhance TAG clearance from the blood stream. The other is thought to be reduced hepatic VLDL secretion (Fukuda *et al.*, 1991). It is not possible to tell conclusively which of these two mechanisms if any predominates in the present study, although insulin concentrations were lower in the LIST compared to both the Walk and Rest trials (main effect trial, P = 0.031 and P = 0.002 respectively). As insulin inhibits muscle LPL activity in the postprandial state (Kiens *et al.*, 1989) the attenuated insulin response in the LIST trial might have resulted in an upregulation of muscle LPL activity and therefore greater TAG clearance compared with the Walk and Rest trials. The lower fasting and postprandial insulin concentrations seen during the LIST trial are indicative of improved insulin sensitivity as a result of exercise. It appears from the literature that it is the energy expenditure not the intensity that is of prime important in eliciting increased insulin sensitivity, however this is still a matter of debate. The mechanisms involved may be associated with exercise induced glycogen depletion and involve effects on insulin stimulated glucose transporters [for review see Borghouts & Keizer, (2000)].

In addition fasting TAG concentrations were lower in the LIST compared with the Rest trial (main effect trial, $P = 0.022$). The lower fasting TAG concentrations seen may be due to the fact that during low and moderate intensity exercise, the muscle derives energy mainly from circulating free fatty acids and from its own TAG store and therefore the TAG muscle store is depleted. This store is restored during recovery by an increase in LPL activity in muscle that enables the tissue to take up circulating TAG more readily than in the nonexercised state. This results in lower fasting TAG concentrations. As endogenous (VLDL) and exogenous (chylomicrons) TAG are cleared by a common saturable pathway (the rate-limiting step of which is hydrolysis of core TAG by LPL), levels of postprandial lipaemia are related to the size of the endogenous TAG pool (O'Meara *et al* , 1992). Therefore the lower fasting TAG levels seen in the LIST trial could have aided increased TAG clearance. The effect of exercise cannot, however be attributed solely to its influence on fasting TAG pool size as the incremental area (above baseline) under the TAG versus time curve was also reduced. Postprandial NEFA concentrations were also found to be higher during the LIST than the Rest trial (main effect trial, $P = 0.027$); as substrate delivery to the liver is the major determinant of VLDL secretion (Sniderman & Cianflone, 1993) this would not be consistent with decreased hepatic secretion of these

lipoproteins after exercise. However NEFA could also have been oxidized in the liver, which would lead to reduced VLDL secretion. As we did not measure hepatic ketone body production (an indicator of hepatic fatty acid oxidation) we cannot make any firm conclusions.

Although the 19% reduction in postprandial lipaemia following the Walk trial did not reach statistical significance (P = 0.064), the average gross energy expenditure of the Walk session (3.1 MJ) was within the range (1.6 – 7.2 MJ, mean 3.4 MJ) shown in previous studies to lower postprandial lipaemia following a single bout of continuous aerobic exercise (Petitt & Cureton, 2003). It is therefore reasonable to suggest that the reduction in lipaemia is likely to be physiologically important. Also if a greater number of participants had performed the Walk session (resulting in greater power in the statistical analysis) a significant reduction would have been more likely to have been seen.

The 6% greater attenuation in postprandial lipaemia following the LIST exercise trial in comparison with the Walk exercise trial may be explained by two factors, working individually or more likely in combination. The duration of the LIST sessions were on average 5 min longer than the Walk sessions. Also, although every attempt was made to match the exercise intensity elicited by the two different exercise modalities, the intensity was 71.8 ± 1.6 % of peak $\dot{V}O_2$ in the LIST trials compared with 61.6 ± 1.0 % in the Walk trials. The significantly elevated lactate, heart rate, and % HR max values in the LIST trials confirm that the exercise intensity maintained was higher than that in the Walk trials. The fact that both the exercise duration and intensity were greater in the LIST trials suggests the energy expenditure was also higher, and the quantity of energy expended has be shown to be an important determinant of the extent to which TAG is lowered following exercise (Tsetsonis & Hardman, 1996b; Tsetsonis & Hardman, 1996a) and to increase insulin sensitivity leading to reduced fasting and postprandial insulin concentrations (Borghouts & Keizer, 2000).

It is important to note however, that when the RPE ratings were examined there were no differences in feelings of fatigue between the two types of exercise session. This is of interest because it suggests that for the same subjective effort the exercise mode closest to games type activity (the LIST trial) produces greater reductions in postprandial lipaemia than steady state exercise. It is also important to be aware that

the time given to complete both the cruises and jogs in the adapted LIST protocol used in this study were increased greatly from those in the original protocol. This was necessary to reduce the overall intensity of the LIST sessions. However, there was a limit to how much speeds could be reduced before participants had to walk. Therefore in an actual game/match of hockey, rugby or soccer the exercise intensity, and as a result the energy expended, would almost certainly be even greater. This could potentially result in an even greater reduction in postprandial lipaemia than that seen in this study. Of course it also has to be recognized that the intermittent nature of the LIST protocol (including 40 maximal sprints) may artificially elevate many of the markers, which are used to indicate exercise intensity during steady state exercise and may make them inappropriate indicators during intermittent exercise involving maximal sprinting. It is also possible that the participants enjoyed the LIST sessions more than the walk sessions due to the variety of different speeds that participants were required to walk and run at and the greater level of concentration this required. This may have resulted in lower RPE ratings.

In conclusion the findings of this study indicate that games activity can reduce postprandial lipaemia and therefore can be recommended as an appropriate mode of exercise with which to meet recommended activity guidelines.

Chapter 7: Effect of Exercise on Postprandial Lipaemia in Adolescent Boys

7.1 Introduction

Although the clinical manifestations of coronary heart disease are not evident until adulthood, the process leading to coronary atherosclerosis is initiated during childhood and adolescence (McGill *et al.*, 2000). While physical activity or regular exercise has been shown to elicit a beneficial effect on adverse blood lipid and lipoprotein profiles in adults it is unclear if this relationship exists in children (Tolfrey *et al.*, 2000; Rowland, 2001). In Chapter 5 the relationship between cardiovascular fitness and blood lipid profile in adolescents was examined. Although peak $\dot{V}O_2$, sex and waist circumference were found to be important predictors of lnHDL cholesterol and the total cholesterol/HDL cholesterol ratio these three predictor variables explained only 17.1% of the variation in lnHDL cholesterol concentrations and 12.6% of the variation in the total cholesterol/HDL cholesterol ratio. The relatively low percentage of variance in the blood lipid values explained by these three predictors maybe due to the large number of confounding variables (for example the use of risks factors for CHD rather than morbidity and mortality and the wide maturity range of participants). These data suggest that it would be useful to find additional measures with which to examine the effect of physical activity or regular exercise on lipid-lipoprotein profile and metabolism in children and adolescents. One method that has been used previously with adults is to examine the effect of exercise on postprandial lipaemia. There are currently no studies investigating the effect of exercise on postprandial lipaemia in children or adolescents. There is evidence to suggest that as children and adolescents grow and mature their physiological responses to physical activity and exercise vary [for review see Boisseau & Delamarche (2000)], therefore their responses to exercise may differ from those of adults. In addition, reducing postprandial lipaemia even in young people could potentially slow atherogenic progression. Therefore, it is important to investigate what effect exercise has on postprandial lipaemia in children.

The study presented in this chapter sought to investigate the effect of exercise on postprandial lipaemia in adolescents. To date the majority of studies investigating the effect of exercise on blood lipid profile and lipid metabolism in adults have included

exercise modes such as continuous stationary cycling or treadmill running. This may be due to the fact that the intention of the first physical activity guidelines (American College of Sports Medicine, 1978) was to improve fitness and in so doing improve health and there is compelling research evidence to suggest that these modes of exercise improve cardiovascular fitness. However, these modes of exercise may be inappropriate for use with children especially as the activity performed by children has been found to be transitory, with the median duration of an activity at any level (low, high, medium) lasting only 6 s. Evidence suggests that even activity events of low intensity are brief, with a median duration of 6 s and lasting no longer than 22.5 min (Bailey *et al.*, 1995). Thus the tempo of children's physical activity was shown to be one of rapid change: very high short bursts of intense activities were interspersed with brief, but variable, intervals of activities of low and moderate intensity. It is necessary to find exercise programmes involving activities, which can be easily sustained by children over a long period of time (this may include games activities such as hockey, rugby and soccer).

Therefore the aim of the present study is to investigate the effect of continuous modes of exercise such as walking and intermittent modes of exercise such as games activity has on postprandial lipaemia in adolescents. It is hypothesised that since both continuous and intermittent modes of exercise (see Chapter 6) have been shown to attenuate postprandial lipaemia in adults the same relationship will be seen in adolescents.

7.2 Methods

7.2.1 Participants

Nineteen, healthy, recreationally active adolescent males volunteered to take part in this study, which was approved by the Loughborough University Ethical Advisory Committee. Before any testing took place all participants and their primary caregivers had the procedures and risks associated with involvement in the study explained to them (verbally and in writing). Written assent from each participant and consent from their primary caregiver was then obtained. Participants 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 < 160/95 mmHg, and had a BMI < 35 kg/m^2. Participants were randomly assigned to either the Walk exercise group (n = 10) or the Loughborough Intermittent Shuttle Test (LIST) exercise group (n = 9). The LIST was used in this study to simulate games activity and was a modified version of the test reported by Nicholas and colleagues (2000). The physical characteristics of the participants are shown in Table 7.1.

Table 7.1 Physical characteristics of participants

	Walk Group n = 10	LIST Group n = 9
Age (years)	15.3 ± 0.1	15.4 ± 0.1
Height (cm)	177.1 ± 1.7	177.5 ± 1.2
Body Mass (kg)	63.4 ± 1.1	59.8 ± 1.0
BMI	20.3 ± 0.5	19.0 ± 0.3
Sum of Skinfolds (mm)	40.9 ± 7.3	40.9 ± 1.4
Waist/Hip Ratio	0.77 ± 0.01	0.77 ± 0 01
Systolic Blood Pressure (mmHg)	105 ± 2	112 ± 4
Diastolic Blood Pressure (mmHg)	69 ± 4	64 ± 3
Tanner Stage Genital – median (range)	4 (3 – 5)	4 (4 – 5)
Tanner Stage Pubic Hair – median (range)	5 (3 – 5)	5 (4 – 5)
Peak $\dot{V}O_2$ (ml.kg^{-1}.min^{-1}) - Treadmill	44.8 ± 3.4	51.1 ± 2.5
Peak $\dot{V}O_2$ (ml.kg^{-1}.min^{-1}) - Predicted (MSST)	48.0 ± 3.6	47.9 ± 1.2

Values are mean ± SEM

Abbreviation: MSST = Multistage Shuttle Run Test

7.2.2 Anthropometry

Height and body mass were measured using standard methods (see Section 3.3.1). Skinfold thicknesses were measured at four sites (triceps, biceps, subscapular and supra-iliac) using callipers, according to the protocol described by Cameron (1984) (see Section 3.3.2). BMI was calculated as the body mass in kilograms divided by the square of the height in metres. Waist circumference was taken as the minimum diameter measured between the iliac crests and lower ribs. Hip circumference was measured at the level of greatest protrusion of the buttocks and taken as the maximum diameter (see Section 3.3.2).

7 2 3 Preliminary Tests

A series of preliminary tests were completed prior to the main trials. Each participant was asked to make a self-assessment of sexual maturity using a five-point scale described by Tanner (1962) to assess the development of genitalia and pubic hair (see Section 3.3.4). Blood pressure was measured by auscultation using a mercury sphygmomanometer (see Section 3.3.3). Peak oxygen uptake was measured directly using an incremental, uphill treadmill walking test to exhaustion using the Douglas bag method. This test was also used to establish the relationship between oxygen uptake, speed and gradient in order to set the gradient necessary to elicit 60% of their peak $\dot{V}O_2$ at their self-selected speed for the Walk main trial (see Section 3.3.9). In addition peak $\dot{V}O_2$ was estimated using a progressive multistage shuttle run test (MSST) (Ramsbottom *et al*, 1988) (see Section 3.3.5). Individuals also performed a familiarisation session. This was in order that they became accustomed with the Walk and LIST protocols and to set and check the exercise intensity for the main trials. An attempt was made to set the exercise intensity for both exercise modes at an average of 60% $\dot{V}O_2$ peak over each block. Participants in the Walk group were required to perform 4 x 15 min blocks of exercise. This replicated the amount of exercise performed during the main trials. The LIST protocol required participants to repeat a pattern of exercise, which consisted of a walk, sprint, run ("cruise" at 70% of $\dot{V}O_2$ peak) and jog (at 40% of $\dot{V}O_2$ peak) over a marked 20 m distance in a sports hall. This pattern was repeated 10 times forming one "block" of exercise (see Section 3.4.2). Participants in the LIST group performed 2 – 4 blocks of exercise; depending on the time it took to become familiar with the exercise protocol and to achieve the lowest possible intensity without the running speeds becoming so slow participants had to walk.

7.2.4 Study Protocol

It was felt that it would be too demanding for the adolescents who participated in this study to complete three main trials. The trials were performed during school hours, therefore participants were absent from lessons for those periods. Participants were also required to control their physical activity levels and their diet before each main trial (described below). This required a considerable amount of effort on their part. As a result each participant took part in two main trials: a Rest trial and an exercise trial (either Walk or LIST) a minimum of 7 days apart, in a randomised order. During

the Rest trial participants refrained from exercise on day one of the experiment and then performed an oral fat tolerance test on the morning of day two. During the exercise trials on the afternoon of day one, participants performed a 15 min warm-up on a treadmill consisting of 5 min of walking at a 1% gradient, at the speed maintained during the peak $\dot{V}O_2$ test, 5 min of stretching and 5 min of walking at a speed and gradient selected to elicit 60% of peak $\dot{V}O_2$. Participants then completed either four blocks of treadmill walking or four blocks of the LIST, with 3 min rest between each block. The next morning (day two) participants attended the laboratory for a fat tolerance test. Exercise was completed at the same time of day (between 3:30 and 5 pm) in both exercise trials so that the time interval between the end of the exercise sessions and beginning of the fat tolerance tests was similar in both exercise trials (≈16 h). Participants performed all exercise trials in pairs.

Participants were asked to refrain from physical activity for the 2 days before the commencement of each trial and also to record their food and drink intake during the 2 day period before the oral fat tolerance test of their first trial. They were asked to replicated this diet for the subsequent trial.

On day two of each trial participants underwent an oral fat tolerance test (see Section 3.4.3). Blood was sampled (fasting, 0.5, 0.75, 1, 3, 4 and 6 h, see Section 3.5) and either analysed immediately or stored for later analysis (see Section 3.6). Expired air collections were also made (fasting, 2.5 and 5.5 h, see Section 3.4.5). The test meal employed was a high fat, mixed meal comprising of cereal, fruit, nuts, chocolate and whipping cream, with the whipping cream being the main contributor to the fat load. The meal provided 1.25 g fat, 1.07 g carbohydrate and 0.20 g protein per kg body mass (see Section 3 4 4)

7.2.5 Statistical Analysis

The 6 h area under the plasma concentration versus time curves for TAG and glucose were calculated using the trapezium rule. The incremental area under the curve for TAG was calculated using the same method after correcting for baseline concentrations. Fasting total cholesterol, HDL cholesterol, TAG and glucose concentrations and the area under the curve values for TAG and glucose in the two different groups were compared between trials using Student's t-tests for correlated

means. Two-way ANOVA (trial - time) was used to determine differences between trials and over time for postprandial plasma concentrations of TAG and glucose as well as for RER, energy expenditure, carbohydrate and fat oxidation. When there was a main effect of time, differences between individual means were assessed using Student's t-tests with a Bonferroni adjustment to establish were differences lay. Statistical significance was accepted at the $P < 0.05$ level. Data were analysed using the Statistical Package for Social Science (SPSS) software version 11.0 for windows (SPSS Inc, Chicago, IL). Data are presented as means ± standard error of the mean (SEM).

7.3 Results Walk Group

7.3.1 Reponses During Exercise Trial

Data from the Walk trial are presented in Table 7.2. Averages over the four blocks of exercise are given for heart rate, % HRmax, % peak $\dot{V}O_2$, RPE, and block time. Lactate concentrations were based on an average from two measurements (post block 1 and post block 4). The mean gross energy expenditure for the Walk was 2.0 ± 0.1 MJ or 32.3 ± 2.3 $kJ.kg^{-1}$ body mass.

Table 7.2 Lactate, heart rate, percentage of maximum heart rate (%HRmax), percentage of peak oxygen uptake (% peak $\dot{V}O_2$), rate of perceived exertion (RPE) and time to complete each exercise block (Block time) during the Walk trial

	Walk Group
Lactate ($mmol.l^{-1}$)	2.0 ± 0.3
Heart Rate ($beats\ min^{-1}$)	143 ± 3
% HR max	73.7 ± 1.4
% Peak $\dot{V}O_2$ Treadmill	58.9 ± 7.1
RPE Median (range)	11 (9 – 13)
Block time (min:s)	15:00 ± 00:00

Values are means ± SEM, $n = 10$

7.3.2 Blood Concentrations in the Fasted State

Blood concentrations in the fasted state are shown in Table 7.3. There was no difference in fasting total cholesterol, HDL cholesterol, TAG or glucose between the Rest and Walk trials.

Table 7.3 Fasting concentrations of total cholesterol, HDL cholesterol, TAG and glucose in the Rest and Walk trials

	Rest	Walk
Total Cholesterol ($mmol.l^{-1}$)	3.50 ± 0.10	3.50 ± 0.17
HDL-Cholesterol ($mmol.l^{-1}$)	0.89 ± 0.07	0.90 ± 0 05
TAG ($mmol.l^{-1}$)	1.09 ± 0.12	0.93 ± 0.08
Glucose ($mmol.l^{-1}$)	4.46 ± 0.14	4.27 ± 0.15

Values are mean ± SEM, $n = 10$
(no significant differences)

7.3.3 Estimated Change in Plasma Volume

Changes in plasma volume over the period of observation were small (Rest, -1.4 ± 1.0 %; Walk, -2.24 ± 2.3 %) and did not differ between trials, therefore were not corrected in this respect.

7.3.4 Postprandial Blood Responses to the Fat Tolerance Tests

Plasma TAG responses to the test meals and the total and incremental areas under the TAG concentration versus time curves are shown in Figure 7.1. The postprandial TAG concentrations were not statistically lower during the Walk than the Rest trial (main effect trial, $P = 0.062$; main effect time, $P < 0.001$; interaction trial - time, $P = 0.135$). While the total area under the TAG curve was reduced by 14% when the Walk trial was compared with the Rest trial this change was not statistically significant (main effect trial, $P = 0.064$). The corresponding values for the areas under the incremental curves were 8% and $P = 0.835$ respectively.

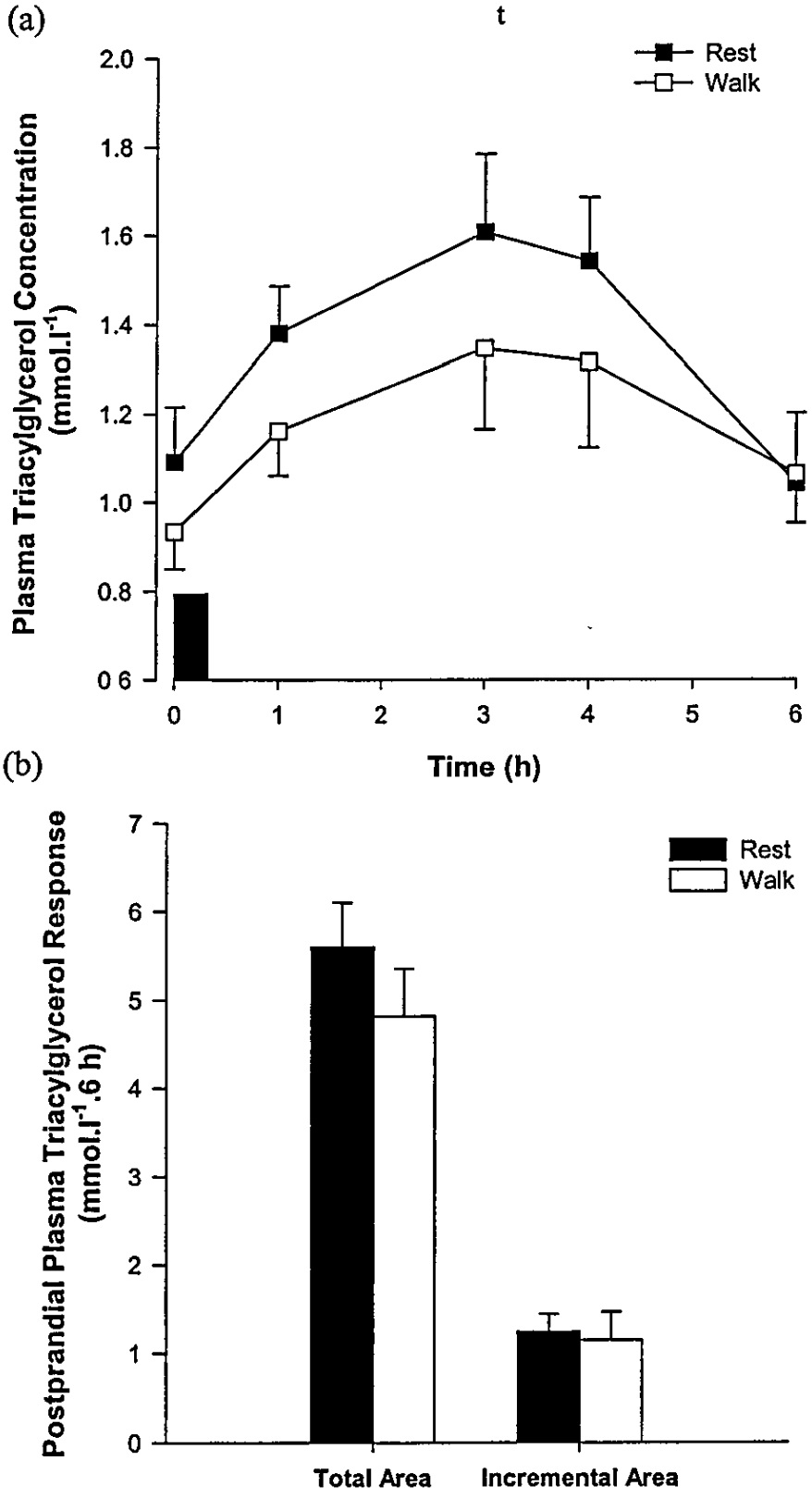

Figure 7.1 Fasting and postprandial TAG concentrations for Rest and Walk trials (a), black rectangle indicates consumption of test meal (main effect trial, $P = 0.062$; ^{t} main effect time, $P < 0\,001$, interaction time - trial, $P = 0.135$). Total and Incremental areas under the TAG concentration versus time curve (b) There were no significant differences (main effect trial, total area, $P = 0.064$; incremental area $P = 0.835$) Values are mean ± SEM, $n = 10$

The glucose responses to the test meals are shown in Figure 7.2. There was no statistical difference between postprandial glucose concentrations in the Rest or the Walk trial (main effect trial, n.s.; main effect time, $P < 0.001$; interaction trial - time, n.s.). The total area under the concentration versus time curve for glucose did not differ between trials (Rest 18.24 ± 0.44 mmol.l^{-1}.6 h and Walk 18.23 ± 0.49 mmol.l^{-1}.6 h).

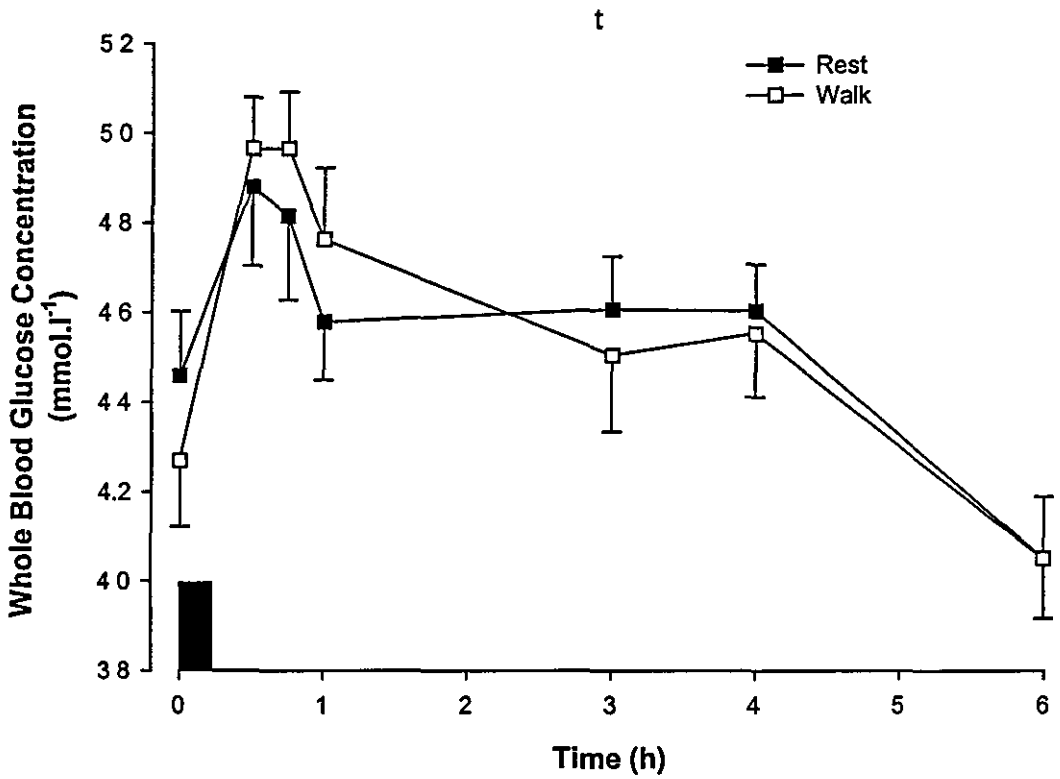

Figure 7.2 Fasting and postprandial glucose concentrations for Rest and Walk trials, black rectangle indicates consumption of test meal (main effect trial, n.s.; ^{t} main effect time, $P <$ 0 001; interaction trial – time, n.s.). Values are mean ± SEM, $n = 10$

7.3.5 Substrate Utilization and Energy Expenditure

Fasting and postprandial values for energy expenditure, respiratory exchange ratio (RER), fat oxidation and carbohydrate oxidation are shown in Table 7.4. No time trial interactions were found for any of these variables. A main effect of time was seen for RER, fat oxidation and carbohydrate oxidation. RER values were lower at 5.5 h compared to fasting values (main effect time, $P = 0.035$). Fat oxidation was

found to be higher at 2.5 h then at fasting (main effect time, P = 0.005) and carbohydrate oxidation lower at 2.5 h then at fasting (main effect time, P = 0.024).

Table 7.4 Substrate utilization and energy expenditure in the fasted and postprandial states for the Walk Group

	Fasting	2.5 h	5 5 h	Mean
Energy Expenditure (kJ.h^{-1})				
Rest	349 ± 16	339 ± 25	328 ± 23	339 ± 21
Walk	344 ± 14	368 ± 17	355 ± 13	355 ± 15
RER				
Rest	0.92 ± 0.05	0.83 ± 0.01	0.81 ± 0.02	0 85 ± 0.03
Walk	0.88 ± 0 02	0.82 ± 0.01	0.84 ± 0.02 t	0.85 ± 0.02
Fat Oxidation (g.h^{-1})				
Rest	4.80 ± 0.35	5.03 ± 0.52	5 43 ± 0.68	5.09 ± 0.52
Walk	3.54 ± 0.58	5.77 ± 0.46 t	4.89 ± 0.66	4.73 ± 0.57
Carbohydrate Oxidation (g.h^{-1})				
Rest	9.37 ± 0.59	8.21 ± 1.13	6.73 ± 1.34	8.11 ± 1.02
Walk	11.89 ± 1.51	8.23 ± 1.11 t	9.46 ± 1.60	9.86 ± 1.41

Values are mean ± SEM, n = 10

t main effect time, significant difference between RER at fasting and 5.5 h (main effect time, P = 0.035), between fat oxidation at fasting and 2.5 h (main effect time, P = 0.005) and in carbohydrate oxidation between fasting and 2. 5 h (main effect time, P = 0.024)

7.4 Results LIST Group

7.4.1 Reponses During Exercise Trial

Data from the LIST trial are presented in Table 7.5. Averages over the four blocks of exercise are given for heart rate, % HR max, % peak $\dot{V}O_2$, RPE, and block time. Lactate concentrations were based on an average from two measurements (post block 1 and post block 4). The energy expenditure for the LIST was not calculated due to

the methodological difficulties in calculating energy expenditure in non steady state exercise when the RER is above unity.

Table 7.5 Lactate, heart rate, percentage of maximum heart rate (%HRmax), percentage of peak oxygen uptake (% peak $\dot{V}O_2$), rate of perceived exertion (RPE) and time to complete each exercise block (Block time) during the LIST trial

	LIST Group
Lactate (mmol.l^{-1})	2.4 ± 0.7
Heart Rate (beats min^{-1})	161 ± 4
% HR max	80.3 ± 1.7
% Peak $\dot{V}O_2$ Treadmill	68.8 ± 2.3
RPE Median (range)	12 (6 – 18)
Block time (min:s)	18:29 ± 00:08

Values are means ± SEM, $n = 9$

7.4.2 Blood Concentrations in the Fasted State

Blood concentrations in the fasted state are shown in Table 7.6. There was no difference in fasting total cholesterol, HDL cholesterol or glucose between the Rest and LIST trials, however fasting TAG concentrations were found to be lower in the LIST trials (main effect trial, $P = 0.001$).

7.4.3 Estimated Change in Plasma Volume

Changes in plasma volume over the period of observation were small (Rest 0.5 ± 2.4 %; LIST, 1.7 ± 1.7 %) and did not differ between trials, therefore concentrations were not corrected in this respect.

Table 7.6 Fasting concentrations of total cholesterol, HDL cholesterol, TAG and glucose in the Rest and LIST trials

	Rest	LIST
Total Cholesterol ($mmol.l^{-1}$)	3.19 ± 0.06	3.16 ± 0.08
HDL-Cholesterol ($mmol.l^{-1}$)	0.91 ± 0.10	0.89 ± 0 09
TAG ($mmol.l^{-1}$)	1.11 ± 0.15	0.92 ± 0.12 ^{T}
Glucose ($mmol.l^{-1}$)	4.46 ± 0.09	4.29 ± 0.14

Values are mean ± SEM, $n = 9$

^{T} Significantly different from Rest (main effect trial, $P = 0.001$)

7.4 4 Postprandial Blood Responses to the Fat Tolerance Tests

Plasma TAG responses to the test meals and the total and incremental areas under the TAG concentration versus time curves are shown in Figure 7.3. The postprandial TAG concentrations were lower during the LIST than the Rest trial (main effect trial, $P = 0.010$; main effect time, $P < 0.001$; interaction trial - time, $P = 0.135$). In addition both the total and the incremental area under the plasma TAG versus time curves were lower in the LIST then the Rest trial (reduced by 26%, main effect trial, $P = 0.006$; reduced by 46%, main effect trial, $P = 0.013$ respectively).

The glucose responses to the test meals are shown in Figure 7.4. There was no difference between postprandial glucose concentrations in the Rest or the LIST trial (main effect trial, n.s.; main effect time, $P < 0.001$; interaction trial - time, n.s.). The total area under the concentration versus time curve for glucose did not differ between trials (Rest 18.08 ± 0.33 $mmol.l^{-1}$.6 h and LIST 17.83 ± 0.37 $mmol.l^{-1}$.6 h).

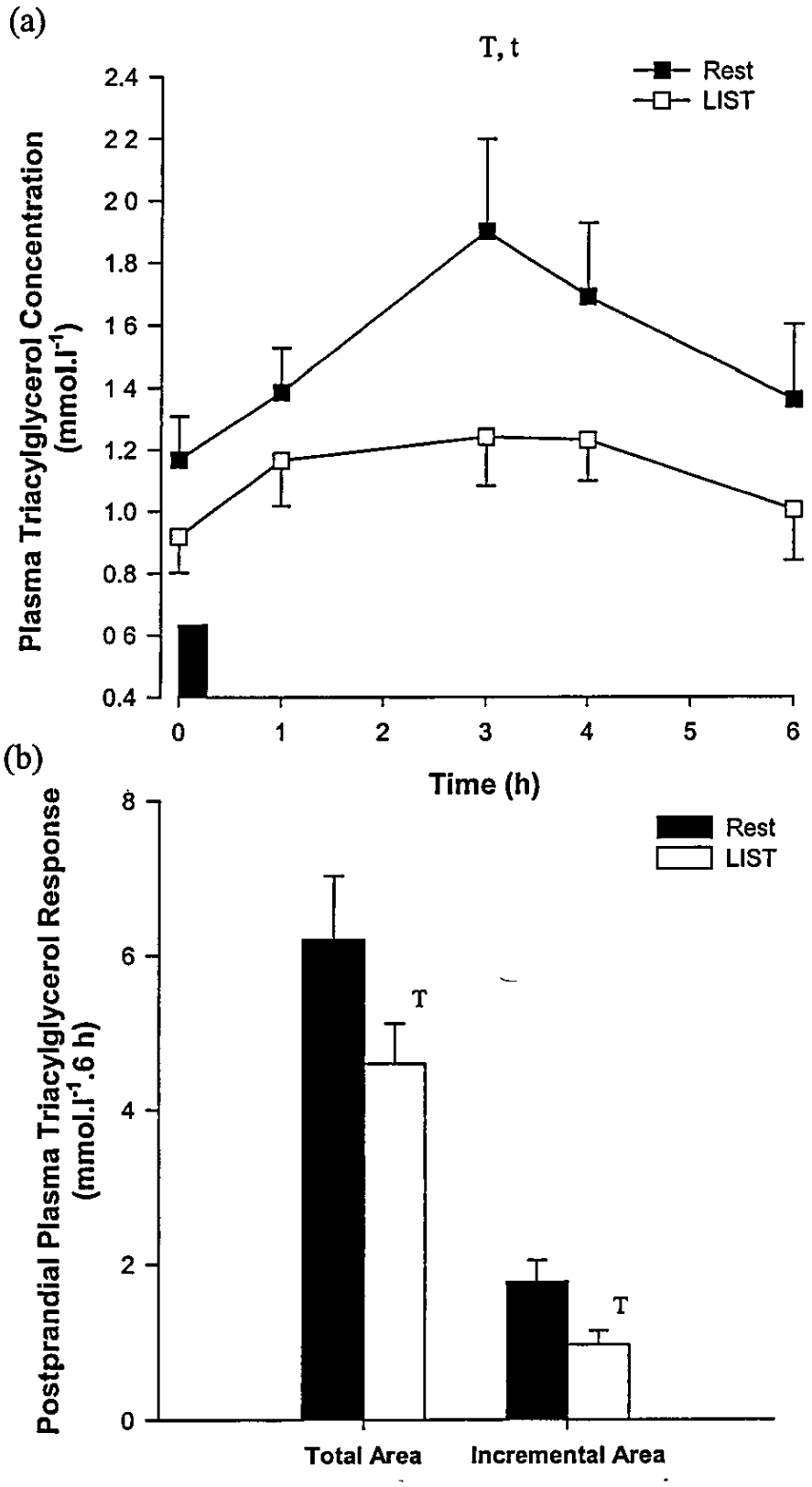

Figure 7.3 Fasting and postprandial TAG concentrations for Rest and LIST trials (a), black rectangle indicates consumption of test meal (^{T} main effect trial, P = 0 010; ^{t} main effect time, $P < 0.001$; interaction trial - time, n.s). Total and incremental areas under the TAG concentration versus time curve (b) ^{T} Significantly different from Rest (main effect trial, total area, $P = 0.006$; incremental area, $P = 0$ 032) Values are mean ± SEM, $n = 9$

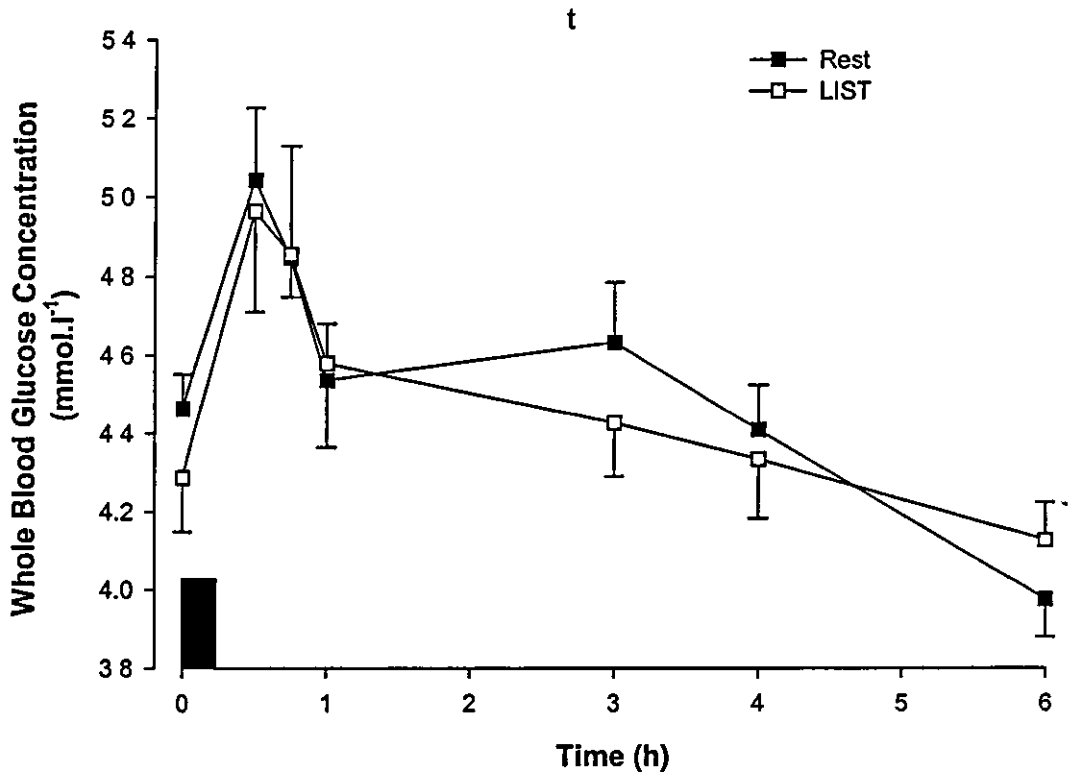

Figure 7.4 Fasting and postprandial glucose concentrations for Rest and LIST trials, black rectangle indicates consumption of test meal (main effect trail, n.s.; ^{t} main effect time, $P < 0.001$; interaction trial - time, n.s.). Values are mean ± SEM, $n = 9$

7.4.5 Substrate Utilization and Energy Expenditure

Fasting and postprandial values for energy expenditure, respiratory exchange ratio (RER), fat oxidation and carbohydrate oxidation are shown in Table 7.7. No main effects of trial, time or time trial interactions were found for any of these variables

Table 7.7 Substrate utilization and energy expenditure in the fasted and postprandial states for the LIST group

	Fasting	2.5 h	5.5 h	Mean
Energy Expenditure ($kJ.h^{-1}$)				
Rest	342 ± 12	365 ± 16	350 ± 14	352 ± 14
LIST	320 ± 47	381 ± 19	355 ± 8	352 ± 25
RER				
Rest	0.88 ± 0.03	0.88 ± 0.02	0.84 ± 0.02	0.87 ± 0.02
LIST	0.82 ± 0.02	0.81 ± 0.02	0.83 ± 0.03	0.82 ± 0.02
Fat Oxidation ($g.h^{-1}$)				
Rest	3.70 ± 0.83	3.71 ± 0.63	5.00 ± 0.69	4.14 ± 0.72
LIST	4.80 ± 0.91	6.36 ± 0.73	5.33 ± 0.82	5.50 ± 0.82
Carbohydrate Oxidation ($g.h^{-1}$)				
Rest	11.43 ± 1.96	12.73 ± 1.53	8 93 ± 1.08	11.03 ± 1.52
LIST	7.68 ± 1.84	7.69 ± 1.89	8.48 ± 1.74	7.95 ± 1.82

Values are mean ± SEM, $n = 9$

main effect trial, n s , main effect time n.s.; interaction trial – time n.s. for all variables

7.5 Discussion

The main finding in the present study was that a single session of intermittent games activity performed 16 h prior to an oral fat tolerance test reduced postprandial lipaemia in a group of young adolescent males. Although the uphill walking also reduced TAG concentrations the difference was not found to be significant at the P < 0 05 level (main effect trial, $P = 0.062$). The total area under the plasma TAG versus time curve was reduced by 26% as a result of the games activity and 14% as a result of the uphill walking. The reduction seen in the Walk group was not statistically significant (main effect trial, $P = 0.064$). As there were two separate exercise groups in this study any comparisons made between the two exercise modes are made with caution.

To the knowledge of the author this is the only study that has examined the effect of exercise on postprandial lipaemia in adolescents. The findings of this study correspond with those of the study described in Chapter 6. Unfortunately due to the use of capillary rather than venous blood samples in this study we were unable to measure insulin and NEFA concentrations. However, it is likely that exercise reduces postprandial TAG concentrations by the same mechanisms as in adults, that is enhanced TAG clearance in the blood stream due to increased LPL activity in the skeletal muscle (Oscai *et al*, 1990) and/or reduced hepatic VLDL secretion (Fukuda *et al.*, 1991). Due to ethical and methodological constraints there is currently limited data available on the metabolic and hormonal responses to exercise in children and adolescents. Despite this, it appears that in children there is an age-dependent response to exercise when compared to that of adults. Two major differences have been observed when comparing the responses of young individuals and adults to prolonged exercise: the first is children have lower muscle glycogen stores (Eriksson *et al.*, 1973), which leads to earlier depletion than is seen in adults; the second is that children have shown a greater utilisation of fat stores (Eriksson, 1980). These factors are unlikely to influence the exercise-induced reduction in postprandial TAG, as in adults it appears to be independent of the type of substrate utilized during exercise. In a study by Malkova and colleagues (1998) substrate utilisation was manipulated by administration of acipimox (which suppresses fatty acid release from adipose tissue) one hour before a 90 min session of moderate exercise. Acipimox was found to increase carbohydrate oxidation and reduced fat oxidation during exercise, when compared with the same exercise session without prior acipimox ingestion. Despite this the reduction to subsequent postprandial TAG concentrations was the same for both exercise sessions (Malkova *et al.*, 1998).

Although the 14% reduction in postprandial lipaemia following the Walk trial did not reach statistical significance (main effect trial, $P = 0.064$), the average gross energy expenditure of the Walk session (2.0 MJ) was within the range (1.6 – 7.2 MJ, mean 3.4 MJ) shown in previous studies to lower postprandial lipaemia in adults following a single bout of continuous aerobic exercise (Petitt & Cureton, 2003). This is a similar result as that found in the study described in Chapter 6. It is therefore reasonable to suggest that the reduction in lipaemia is likely to be physiologically important. Also if a greater number of participants had performed the Walk session (resulting in greater power in the statistical analysis) a significant reduction would

almost certainly have been seen. As in the study described in Chapter 6 the 12% greater attenuation in postprandial lipaemia following the LIST exercise session in comparison with the Walk exercise session may be explained by two factors, working individually or more likely in combination. The duration of the LIST sessions were on average 13 min 56 s longer than the Walk sessions. Clearly with hindsight because of the difference in duration between the two exercise sessions it is not possible to discount this as a factor that influenced the observed response. It would have been better if the duration could have been more closely matched. The exercise intensity elicited by the two exercise modalities was higher in the LIST than the Walk sessions (68.8 ± 2.3 % and 58.9 ± 7.1 % of peak $\dot{V}O_2$, respectively). Although it might be tenuous to make direct comparisons between the two difference exercise modes as there were two different exercise groups in this study, lactate concentrations, heart rate, and % HR max values were all higher in the LIST than the Walk group during the exercise sessions. This could suggest that the exercise intensity maintained by participants in the LIST group was higher during the LIST than that maintained by the Walk group in the Walk sessions. The fact that both the exercise duration and intensity were greater in the LIST trials suggests the energy expenditure was also higher, and the quantity of energy expended has been shown to be an important determinant of the extent to which TAG is lowered following exercise (Tsetsonis & Hardman, 1996b; Tsetsonis & Hardman, 1996a). In the study presented in Chapter 6 it was found that 60 min of walking at 60% of peak $\dot{V}O_2$ did not reduce postprandial lipaemia significantly in the young adult participants. Despite this it was important to determine if this dose/volume of exercise could reduce postprandial lipaemia in adolescents as the current physical activity guidelines recommend that adolescents perform at least 60 min of moderate activity per day (Biddle *et al.*, 1998). When the energy expended during the walk exercise session is examined in relation to body weight, the adolescents expended 32.3 ± 2.3 compared to 40.2 ± 1.5 $kJ.kg^{-1}$ body mass expended by the young adults in Chapter 6. This could explain the slightly greater reduction in postprandial lipaemia seen in the young adults as a result of the walk exercise session (19% in the young adults versus 14% in the adolescents)

It is important to note however, that as was found in the study described in Chapter 6 the feelings of fatigue elicited by the two different exercise modalities were similar between groups (as indicated by the RPE ratings). This is of interest because it

suggests that for the same subjective effort the exercise mode closest to games type activity (the LIST trial) produces greater reductions in postprandial lipaemia in the LIST group than steady state exercise did in the Walk group. As with the participants Chapter 6 the time given to complete both the cruises and jogs in the adapted LIST protocol used in this study were increased greatly from those in the original protocol in an attempt to reduce the overall intensity of the LIST sessions (it is important to note that the LIST was developed to simulate games activity in adults not adolescents so if might not be a true represent of the speeds run during games activity in adolescents). However, there was limit to how much speeds could be reduced before participants would have to walk. Therefore in an actual game/match of hockey, rugby or soccer the exercise intensity, and as a result the energy expended, could be even greater. This could potentially result in an even greater reduction in postprandial lipaemia than that seen in this study. As in the study described in Chapter 6 it has to be recognized that the intermittent nature of the LIST protocol (including 40 maximal sprints) may artificially elevate many of the markers, which are used to indicate exercise intensity during steady state exercise and may make them inappropriate indicators during intermittent exercise involving maximal sprinting. As in the study presented in Chapter 6 it is also possible that the participants enjoyed the LIST sessions more than the walk sessions due to the variety of different speeds that participants were required to walk and run at and the greater level of concentration this required. This may have resulted in lower RPE ratings.

There are currently a limited number of studies indicating that exercise has a beneficial influence on the lipid and lipoprotein profile of children or adolescents [for reviews see Armstrong & Simons-Morton (1994) and Tolfrey *et al.*, (2000)]. Therefore the reduction in postprandial lipaemia seen in response to exercise in the group of adolescents in this study is perhaps unexpected. However, when differences in lipid and lipoprotein profile are detected one of the most consistent findings from cross-sectional studies in children and adolescents is that athletic or highly active children have higher levels of HDL cholesterol compared to inactive groups (Rowland, 2001). As HDL cholesterol concentration is a marker for the efficiency of TAG metabolism in the postprandial state (Miesenbock & Patsch, 1992) the higher HDL concentrations seen in these more active children and adolescents could be a result of exercise increasing their ability to metabolise TAG efficiently. As is evident

by the reduction of postprandial lipaemia seen in the group of adolescents who performed the LIST protocol in this present study.

In conclusion the findings of this study indicate that intermittent games type activity can reduce postprandial lipaemia in adolescent boys and therefore can be suggested as an appropriate mode of exercise with which to meet the current recommended activity guidelines for young people.

Chapter 8: General Discussion

8.1 Introduction and Key Findings

The research studies presented in the preceding three chapters examined the effect of exercise on blood lipid-lipoprotein metabolism in both adolescents and young adults. The main findings are summarised below:

1. The results presented in Chapter 5 provide evidence to support the view that it is important for young people to lead an active lifestyle as this could improve their cardiorespiratory fitness levels. It was found that in a group of adolescents, peak $\dot{V}O_2$, sex and waist circumference were found to be predictors of blood lipid-lipoprotein profile. These three predictor variables explained 17.1% of the variation in lnHDL cholesterol concentrations and 12 6% of the variation in the total cholesterol/HDL cholesterol ratio.

2. The main finding in the study presented in Chapter 6 was that a single session of intermittent games activity performed 16 h prior to an oral fat tolerance test reduced postprandial lipaemia in a group of young adult males. The total area under the plasma TAG versus time curve was reduced by 25% and 19% in the LIST and Walk exercise trials respectively when compared to the Rest trial. However, the reduction seen in the Walk trial was not statistically significant (main effect trial, $P = 0.064$). These findings indicate that games activity can reduce postprandial lipaemia and therefore can be recommended as an appropriate mode of exercise with which to meet the current recommended physical activity guidelines.

3. The study described in Chapter 7 examined the effect of exercise on postprandial lipaemia in adolescent males. To our knowledge this is the first study to do so. The findings of this study correspond with those in the study undertaken in young adult males described in Chapter 6. A single session of intermittent games activity performed 16 h prior to an oral fat tolerance test reduced postprandial lipaemia in a group of young adolescent males. The total area under the plasma TAG versus time curve was reduced by 26% as a result of the games activity and 14% as a result of the uphill walking. As in the

study described in Chapter 6 the reduction seen as a result of the uphill walking was not found to be statistically significant (main effect trial, P = 0.064). The findings of this study indicate that intermittent games type activity can be recommended as an appropriate mode of exercise, which adolescents can use in an attempt to meet the current recommended activity guidelines.

The following discussion will try to analyse these points with respect to other research and evaluate what inferences can be made (if any) from these studies

8.2 Epidemiological Investigations

The study conducted in Chapter 5 sought to establish if physical activity or regular exercise might have a beneficial effect on blood lipid and lipoprotein profiles in a population of adolescents. While peak $\dot{V}O_2$, sex and waist circumference were found to be important predictors of lnHDL cholesterol and the total cholesterol/HDL cholesterol ratio in a group of adolescents, these three predictor variables actually explained 17.1% of the variation in lnHDL cholesterol concentrations and 12.6% of the variation in the total cholesterol/HDL cholesterol ratio. However, this still leaves a considerable portion of the variation unexplained. There may be a whole host of reasons for this. It is very important to note that studies examining associations between cardiovascular fitness and CHD in adults have the advantage of using mortality and morbidity as outcome variables, whereas children's studies are restricted to examining risk factors for CHD rather than death or morbidity arising from the disease. It has been suggested that the risk factors for CHD mortality such as high blood pressure, elevated blood lipids and fatness may only account for 50% of eventual coronary mortality in adults and are therefore, a relatively crude measure of coronary health (Thompson & Wilson, 1982). This could be even lower in children and adolescents as these markers of ill health in adults may not be clear markers in young people. This could potentially obscure any relationship between cardiovascular fitness and CHD in young people. The risk factors for CHD may also be affected by growth and maturation. It is also possible that high levels of cardiovascular fitness and therefore a low risk of developing CHD may be largely genetically determined rather than a result of high physical activity levels. All of these factors may mean that while epidemiological investigations are useful in adult populations their efficacy

in distinguishing factors contributing to disease risk in relatively healthy asymptomatic young populations is debateable.

In addition, while taken collectively the studies presented in this thesis suggest that exercise can have a beneficial effect on blood lipids and lipoproteins in both adolescents and young adults, the study described in Chapter 5 is not particularly useful with respect to determining physical activity guidelines. It can give no indication of the duration, intensity or volume of exercise needed to elicit beneficial effects on blood lipid-lipoprotein profile. Also, the possibility that it will provide information about the existence of a physical activity threshold, and where this might be, seems unlikely. As a result, the data collected from the population based study described in Chapter 5 seems of limited use in providing evidence on which physical activity guidelines can be generated. However studies in the postprandial state seem an ideal method of providing such information. The study described in Chapter 5 did however confirm that there is a relationship between cardiorespiratory fitness and blood lipid-lipoprotein in adolescents and that it is important for young people to lead an active lifestyle as this could improve their cardiorespiratory fitness levels.

8.3 Intermittent Games Activity and Postprandial Lipaemia

In adults, studies which have investigated the effect of exercise on postprandial lipaemia, have provided information about what duration and intensity of exercise is necessary to produce a beneficial-result, that is to reduce postprandial lipaemia (Tsetsonis & Hardman, 1996b; Tsetsonis & Hardman, 1996a). Such studies have also been used to investigate the effects of different modes of exercise, for example resistance training (Petitt *et al.*, 2003; Burns *et al.*, In Press) on lipid metabolism. In addition, postprandial studies have also recently (Miyashita *et al.*, Unpublished) been used to determine if the current physical activity recommendations of the accumulation of multiple bouts of aerobic activity (minimum duration of 10 min accumulated throughout the day) have beneficial effects on lipid metabolism. It would be useful to have this information for children and adolescents. Especially as the current physical activity guidelines for young people (Sallis, 1994; Biddle *et al*, 1998) are based mainly on data collected in adults. Since there is evidence to suggest that children and adolescents may response differently to exercise and physical activity than adults (Boisseau & Delamarche, 2000) this is unacceptable.

The studies carried out in Chapters 6 and 7 on young male adults and male adolescents respectively, clearly showed that approximately 65 to 75 minutes of intermittent games activity performed 16 hours before ingestion of a high fat meal, reduced the lipaemic response following the meal. As the process of atherosclerosis may well be linked to the transit time of lipids in the body the fact that games exercise, mirroring activities that are very popular with the young, reduced postprandial lipaemia is a very important finding. It also adds support to the assertion that young people should exercise on a regular basis.

The results of the two studies presented in Chapters 6 and 7 are remarkably similar. The LIST reduced postprandial lipaemia by 25% in young adults and 26% in adolescents whereas the Walk reduced postprandial lipaemia by 19% in young adults and 14% in adolescents (although this reduction was not found to be statistically significant). The fact that the Walk reduced postprandial lipaemia to a slightly lesser extent in the adolescents than in the young adults may be due to the energy expended. The mean gross energy expenditure expend in the Walk was 3.1 ± 0.2 MJ or 40.2 ± 1.5 kJ kg^{-1} body mass for the adult participants and 2.0 ± 0.1 MJ or 32.3 ± 2.3 $kJ.kg^{-1}$ body mass for the adolescents. The energy expended during the LIST was not calculated so it is difficult to suggest reasons why the reduction in postprandial lipaemia was so similar in both the young adults and adolescents even when the adolescents exercised for approximately 9 min longer (which would suggest that the energy expended by the adolescents would be higher than that expended by the young adults). It is possible that, although the duration was longer the overall energy expended during the LIST sessions by the young adults and adolescents were more closely matched. These results suggest that maturation may not greatly affect postprandial lipid responses to exercise. However it would be useful to examine the effect of exercise on prepubertal children to confirm this view.

Fasting as well as postprandial TAG concentrations were lower in the LIST compared with the Rest trials in both the young adults and adolescents. These lower fasting TAG concentrations are most likely due to the fact that during exercise the TAG muscle store is depleted This store is restored during recovery by an increase in LPL activity in muscle that enables the tissue to take up circulating TAG more readily than in the nonexercised state. This results in lower fasting TAG concentrations. These

lower concentrations of fasting TAG also contribute to the reduction in postprandial lipaemia seen as a result of exercise. Since endogenous (VLDL) and exogenous (chylomicrons) TAG are cleared by a common saturable pathway (the rate-limiting step of which is hydrolysis of core TAG by LPL), levels of postprandial lipaemia are related to the size of the endogenous TAG pool (O'Meara *et al.*, 1992) Therefore, the lower fasting TAG concentrations seen in the LIST trials in both the young adults and adolescents could have aided increased TAG clearance. The reduction in postprandial lipaemia as a result of exercise cannot however, be attributed solely to its influence on fasting TAG pool size as the incremental area (above baseline) under the TAG versus time curve were also reduced in both the young adults and adolescents. Currently the mechanisms responsible for the exercise induced reductions in postprandial TAG concentrations are not completely understood. However it seems likely that exercise reduces postprandial TAG concentration by two mechanisms working either together or separately. One is thought to involve increased LPL activity in the skeletal muscle (Oscai *et al.*, 1990), which may enhance TAG clearance from the blood stream. The other is thought to be reduced hepatic VLDL secretion (Fukuda *et al.*, 1991). It is not possible to tell conclusively, which of these two mechanisms if any predominates in the studies involving the young adults or the adolescents presented in this thesis. Unfortunately due to methodological constraints it was not possible to measure insulin and NEFA concentrations in the adolescents. However, in the young adults lower fasting and postprandial insulin concentrations were found in the LIST compared to both the Walk and Rest trials. This could have resulted in an upregulation of muscle LPL activity and therefore greater TAG clearance in the LIST compared with the Walk and Rest trials as insulin has been shown to inhibit muscle LPL activity in the postprandial state (Kiens *et al.*, 1989). The lower fasting insulin concentrations seen during the LIST trial are indicative of improved insulin sensitivity as a result of exercise. The mechanisms responsible for this improved insulin sensitivity have not yet been fully elucidated but they may involve effects on insulin stimulated glucose transporters as a result of exercise induced glycogen depletion [for review see Borghouts & Keizer, (2000)]. In the young adults postprandial NEFA concentrations were also found to be higher during the LIST than the Rest trial (main effect trial, $P = 0.027$). This could potentially have resulte in increased VLDL secretion from the liver as this is largely determined by NEFA delivery to the liver (Sniderman & Cianflone, 1993). However this NEFA could also have been oxidized in the liver, which would lead to reduced VLDL secretion. As we did not measure hepatic ketone

body production (an indicator of hepatic fatty acid oxidation) we cannot make any firm conclusions.

Interestingly the data from Chapters 6 and 7 suggests that for the same subjective rate of perceived exertion the participants were able to exercise and maintain a higher exercise intensity and thus expend a higher amount of energy during the intermittent games activity exercise sessions than during the uphill walking exercise sessions. This could mean that if a person played rugby for one hour they could potential expend more energy than if they walked for one hour. It is also important to point out that in order to elicit 60% of peak VO_2 during the walk sessions the participants had to walk at a gradient ranging from 5 to 12 % in the young adult participants and from 0 to 9.5% in the adolescent participants. This indicates that it might be difficult in healthy adolescents and young adults who have high cardiorespiratory fitness levels to expend the required amount of energy walking unless the sessions are of high duration.

Clearly the data collected from the studies performed in Chapters 6 and 7 support the assertion that intermittent games activity is an ideal mode of exercise with which adolescents and young adults can achieve current recommended physical activity guidelines [that is, to perform at least 1 h of moderate activity per day (Biddle *et al.*, 1998)].

8.4 Directions for Future Research

1. It is important to discover if postprandial lipaemia in adolescents can be reduced by continuous modes of exercise such as running and walking. Although a 14% reduction was seen as a result of uphill walking at moderate intensity (60% of peak $\dot{V}O_2$) this difference was not found to be statistically significant.

2. Examine the effect of postprandial lipaemia on prepubertal children to determine what effect maturation has on postprandial lipid responses to exercise.

3. Assess the effects of the accumulation of exercise throughout the day on postprandial lipaemia in children and adolescents.

4. Investigate whether well trained children and adolescents have different postprandial responses to sedentary individuals.

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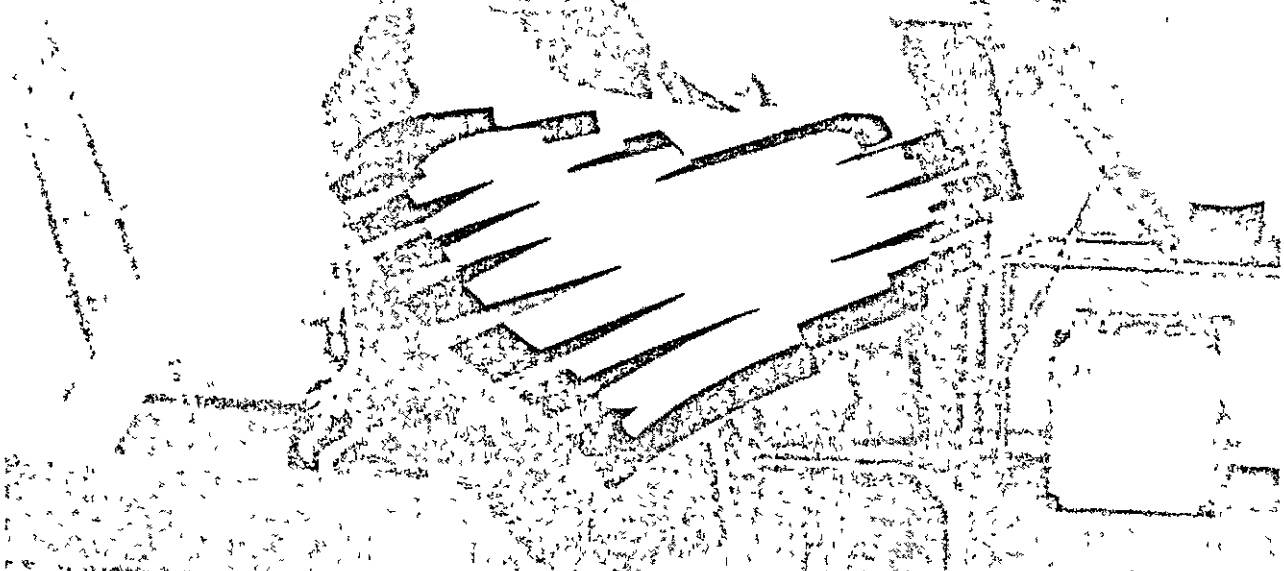
Loughborough University
School of Sport and Exercise Sciences

Interested in helping to fight heart disease?



We are conducting research into exercise and heart disease risk and are looking for men aged 18 to 35 to help. We hope to determine whether games activity such as football or hockey can help decrease a person's risk of developing cardiovascular disease. You would be required to perform two different exercise sessions and we would see how these sessions affect your body's ability to deal with a high fat meal by monitoring changes in various blood constituents.

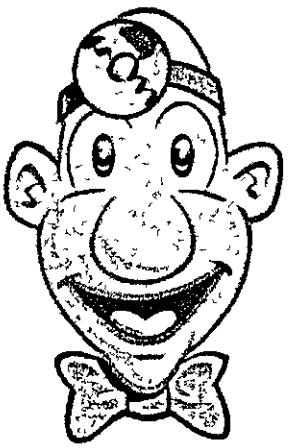
For further information contact Dr John Morris or Laura Barrett

Email: J.G.Morris@lboro.ac.uk telephone no: 01509 226314 room JB1.20
Email: L.A.Barrett@lboro.ac.uk telephone no: 01509 228183 room RR1.05

Study Information

Screening

- Blood pressure
- Saliva test- to measure testosterone
- Height and weight
- Skinfold and circumference measurements - to measure the amount of fat in your body
- Health questionnaire - to make sure you have no health problems which would prevent you from participating in the study
- Self-assessment of maturity

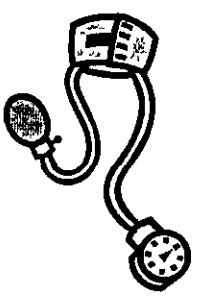


Self-Assessment of Maturity

The reason for this assessment is that young people of the same age can be at very different stages of maturity, e.g. 13-14 year old boys may look slim and slight or tall and thicker-set depending on whether or not they have gone through puberty. It is a better comparison to examine the test results of young people of the same maturity rather than of the same age. The assessment procedure requires you to enter an enclosed room on your own and carefully study some pictures of different stages of development (e.g. genital development and amount of pubic hair). You should then hold-up or pull-down your clothing (there is no need to completely remove clothing) and look in the mirror and decide which picture most closely matches your own stage of development. Write the number of that picture down on the form, place the form in the envelope and seal it. Fully replace all of your clothing before leaving the room and hand the envelope to the person leading the testing.

Preliminary Tests

- Multi stage shuttle test
- Peak oxygen uptake test
- Walk familiarisation or LIST familiarisation



Multi Stage Shuttle Test

This test involves running over a 20m distance. You will need to run in time to an audio signal (a 'bleep') which indicates when you should be at the end of each 20m. You will need to turn at the end of the 20m then begin the next 20m. You will be required to keep time to the 'bleeps' until you can no longer do so. The speed at which the 20m distance should be run increases every 60s.

Peak Oxygen Uptake Test

This test measures the maximum amount of oxygen (the substance in air we require to be able to breath) you can take into your body and use in your muscles to produce energy. This test involves walking on a treadmill at different gradients. A starting speed which you find comfortable will be determined at the beginning of the test and this speed will not change during the test.

During the last minute of each 4 minutes at a particular gradient you will be asked to breath through a mouthpiece so that we can collect the air that you breath out. Then while you are still walking you will place your hand on the rail of the treadmill and a finger prick blood sample will be taken The treadmill gradient will then be increased and you will start the next stage This process will be repeated until you feel you can only keep walking for one more minute. You will indicate this to the tester by raising one finger clearly in the air. An air sample will then be collected during this minute and the test is complete.

Main Trials – (see Figure 1)

- Rest Trial
- Exercise Trial (either 60 min walking or LIST)

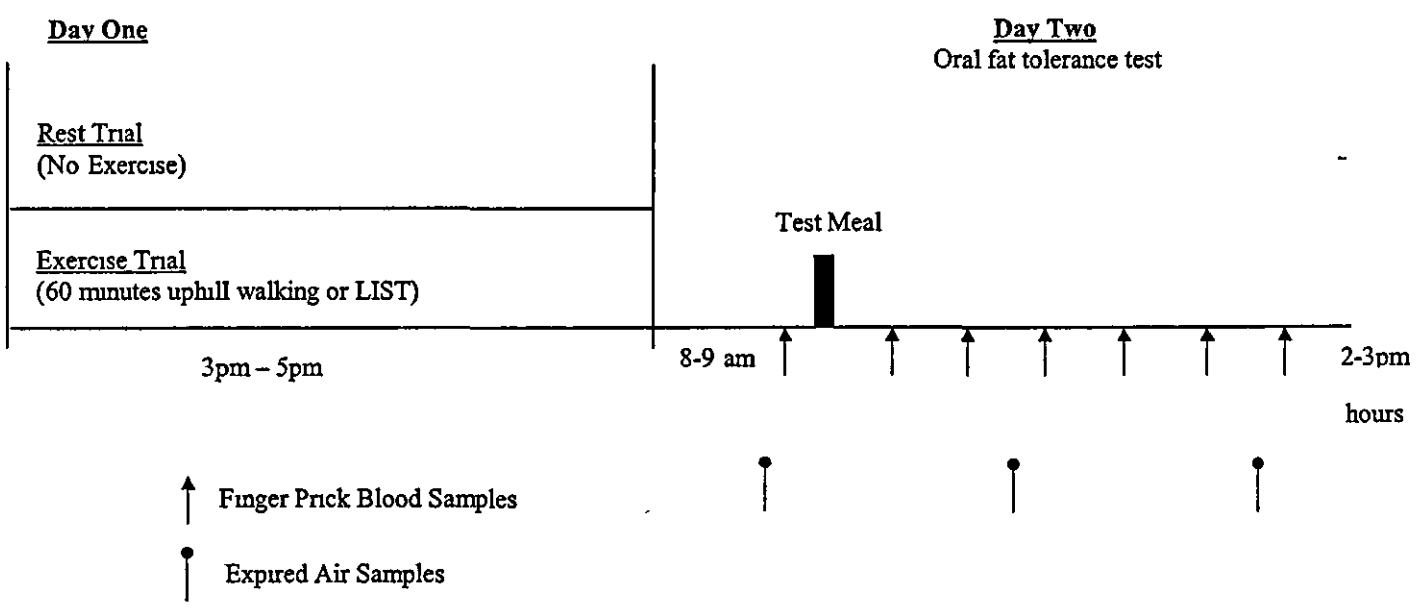


Figure 1

Rest Trial

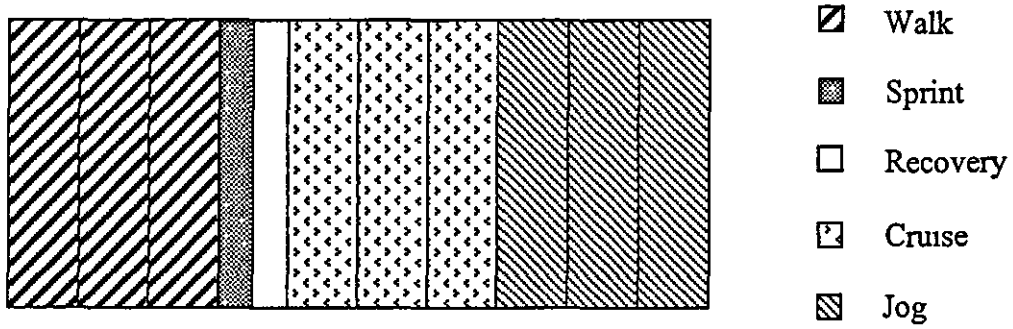
You will need to be as inactive as possible the day before an oral fat tolerance test.

Exercise Trial

You will be asked to perform a 60 min session (4 x 15 min, with 3 min break in between) of either continuous (uphill walking on a treadmill) or intermittent exercise (LIST) the afternoon before an oral fat tolerance test.

The Loughborough Intermittent Shuttle Test

The Loughborough Intermittent Shuttle Test (LIST) will be used to simulate games activity in this study. This test is performed indoors and simulates the activity patterns common in games such as football and hockey, without the contact. This test involves a repeated pattern of walking or running shuttles of 20 m at various speeds (The pattern is shown in Figure 2).



The LIST is conducted over 20 m, which is identified by cone and floor markings.
3 x 20 m at walking pace,
1 x 20 m at maximal speed,
3 x 20 m at a speed determined as cruise pace,
3 x 20 m at a speed determined as jog pace.
THIS SEQUENCE IS REPEATED 10 TIMES = 15 MINUTES OF EXERCISE

Figure 2

Oral Fat Tolerance Test

You will report to the laboratory after an overnight fast and will be asked to lie quietly under a special transparent hood for about 10 minutes whilst your energy needs at rest will be measured. A finger prick blood sample will then be taken.

Next you will be given a high fat meal to eat. This will comprise of whipping cream, apple, banana, sultanas, chocolate drops, brazil nuts, coconut and oats.

Further blood samples (6) and expired air samples (2) will be obtained at intervals during the 6 hour observation period. The concentrations of fat in the blood samples collected will be determined.

Following the meal you will not be allowed to eat or drink anything other than water and you will be asked to rest quietly throughout the 6 hour observation period. You will be allowed to read, watch television/videos.

Time Requirements

- Visit 1 - Multi stage shuttle test - approximately 20/30 min
- Visit 2 - Peak oxygen uptake test - approximately 45 min
- Visit 3 - Walk familiarisation or LIST familiarisation - approximately 1 ½ to 2hr
- Visit 4 - Rest trial + Oral Fat Tolerance Test - On the day before the oral fat tolerance test (see time requirements detailed below) you will need to refrain from physical activity.
- Visit 5 & 6 - Exercise trial + Oral Fat Tolerance Test - On the day before a different oral fat tolerance test (time requirements detailed below) you will come to the University and complete a walking or LIST session in the afternoon - approximately 1 ½ to 2hr

Oral fat tolerance tests - approximately 6 ½ to 7 hours

- Standardisation of food consumption and diet before and during the main trials will require the completion of a food diary (estimated to take 20 min).

You will be required to be as inactive as possible for the 2 days before the start of each trial.

Benefit of the Study

The aim of this study is to try and see if exercise can help your body deal with the fat you eat by clearing it more quickly from your blood system so that it has less time to damage your blood vessel walls.

Possible risks and discomfort:

Two of the tests involve maximal exercise and are therefore demanding. However, you will be encouraged to stop exercising if the demands of the test become unbearable. The finger prick blood sample maybe uncomfortable and may cause your finger to feel a little sore for a day or so

A Comparison of the Effect of Games Activity with that of Continuous Exercise on Postprandial Lipaemia in Children

Young Person's Willingness to Participate Form

Please read the statements below and indicate whether you are willing to participate in this study.

Willingness to Participate Statement:

- I have read the information about the study and the tests involved have also been explained to me. I have had the opportunity to ask questions and I understand what is required of me. I know that I can say that I do not wish to continue with the testing at any time and I do not have to give a reason.

- I agree to take part in the tests (please tick): ☐

Signed: _____ Date: _____

Witnessed by: _____

A Comparison of the Effect of Games Activity with that of Continuous Exercise on Postprandial Lipaemia in Children

Parent/Guardian Consent

- I have been invited to observe procedures.
- I have been given the opportunity to ask questions (please contact Dr John Morris if you have any questions, see below) and I understand what is required from my son.
- I have seen the information sheet and fully understand what the tests entail.
- I give permission for my son (please print your son's name) to be involved in the testing. _____
- Parent/guardian's signature _____
- Parent/guardian's name (please print) _____
- Does your son take any medication? Yes ☐ No ☐
  If yes, please explain:
- Does your son have a medical condition? Yes ☐ No ☐
  If yes, please explain:
- Does your son have any food allergies? Yes ☐ No ☐
  If yes, please explain:

Direct line for Dr John Morris: 01509 226314
Email address for Dr John Morris: J.G.Morris@lboro.ac.uk

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HEALTH SCREEN FOR STUDY VOLUNTEERS

Name or Number .....

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) attending 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 require 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/coordination..... 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. **If you did** smoke or drink would you indicate that on a questionnaire such as this? .... Yes ☐ No ☐

5. **Has any,** otherwise healthy, member of your family under the age of 35 died suddenly during or soon after exercise? .. 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.)

....

....

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6 **What is your ethnic origin?**
(Ethnic origin questions are not about nationality, place of birth or citizenship. They are about colour and broad ethnic group – UK citizens can belong to any of the groups indicated).

White
British ☐
Irish ☐
Any other White background ☐
(please specify).....

Mixed
White & Black Caribbean ☐
White & Black-African ☐
White & Asian ☐
Any other Mixed background ☐
(please specify).....

Asian or Asian British
Indian ☐
Pakistani ☐
Bangladeshi ☐
Any other Asian background ☐
(please specify) .....

Black or Black British
Caribbean ☐
African ☐
Any other Black background ☐

Chinese or other ethnic group
Chinese ☐
Any other ☐
(please specify).....

7. **Do you smoke?**..... ....Yes ☐ No ☐
No per day.....

8. **Do you drink alcohol?**.....Yes ☐ No ☐
Amount per week.....

Additional questions for female participants

(a) Have your periods started yet? .... Yes ☐ No ☐
If yes please answer (b) to (e)
(b) at what age did your periods start (as accurately as you can remember)?
Age.....years and.....months
(c) are your periods normal/regular? .... Yes ☐ No ☐
(d) are you on "the pill"?..... Yes ☐ No ☐
If yes what type?..... .....
(e) could you be pregnant?.....Yes ☐ No ☐

Thank you for your cooperation!

Loughborough University

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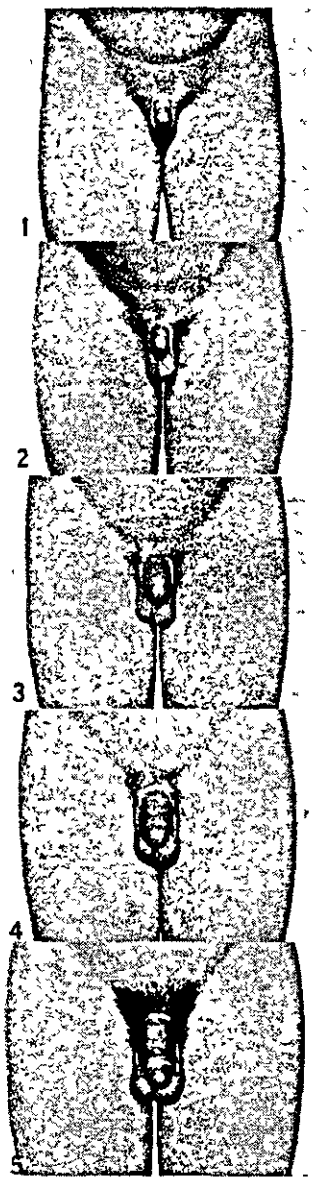
Loughborough University

TANNER STAGES:
MALE GENITAL DEVELOPMENT

The pictures on this page show different stages of development of the genitals of boys (i.e. the testes and scrotum, and penis). A boy passes through each of the five stages shown by these pictures. Please look at each of the pictures and read the sentences next to the picture. Then choose the picture closest to your stage of development and mark an **A** on the picture. Then choose the picture that is next closest to your stage of development and mark a **B** on the picture. In choosing the right picture, look only at the size and shape of the genitals not at the pubic hair.



Stage 1
The testes, scrotum and penis are about the same size and shape as they have always been since you were a child.

Stage 2
The testes and scrotum have become a little larger. The feel of the skin of the scrotum has changed and it is slightly darker. The scrotum, the sack holding the testes, has lowered a bit.

Stage 3
The penis has grown a little in length. The testes and scrotum have grown bigger and dropped lower than in stage 2.

Stage 4
The penis has grown larger and is wider. The glans (the head of the penis) is bigger than before. The testes have grown bigger and are darker.

Stage 5
The penis, scrotum and testes are the size and shape of that of an adult male.

Once you have completed the form, fold it and put it in the envelope provided and seal the envelope.

Your results are completely private and will be treated in complete confidence. No one will know who has filled out the form, as your name will not be on it.

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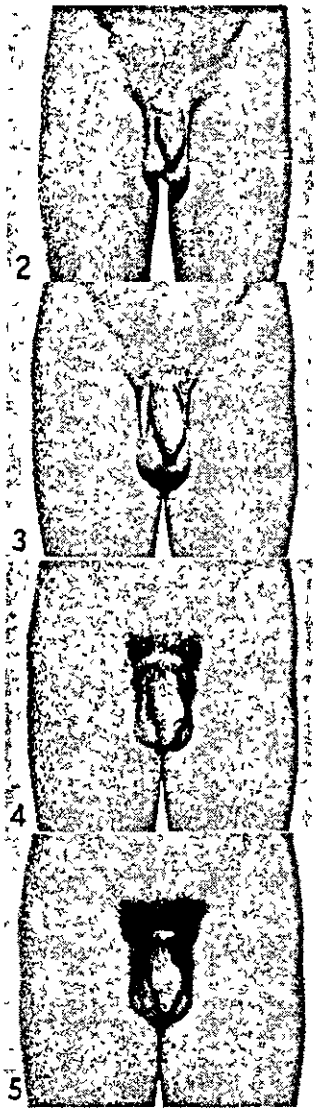
TANNER STAGES:
MALE PUBIC HAIR DEVELOPMENT

The pictures on this page show different stages of development of male pubic hair. A boy passes through each of the five stages shown by these pictures. Please look at each of the pictures and read the sentences next to the picture. Then choose the picture closest to your stage of development and mark an **A** on the picture. Then choose the picture that is next closest to your stage of development and mark a **B** on the picture. In choosing the right picture, look only at the pubic hair and not at the size of the testes, scrotum and penis.

Stage 1
(No picture)



Stage 1
There is no pubic hair at all.

Stage 2
There is a little soft hair. Most of the hair is at the base of the penis. This hair may be straight or a little curly.

Stage 3
The hair is darker in this stage. It is coarser and more curled. It has spread out and thinly covers the area around the penis.

Stage 4
The hair is now as dark as that of an adult man. However, the area it covers is not as large as that of an adult man. The hair has not spread out to touch the thighs.

Stage 5
The hair has spread out to touch the thighs. The hair is now like that of an adult man. It also covers the same area as that of an adult man and has the shape of a triangle (∇).

Once you have completed the form, fold it and put it in the envelope provided and seal the envelope.

Your results are completely private and will be treated in complete confidence. No one will know who has filled out the form, as your name will not be on it.

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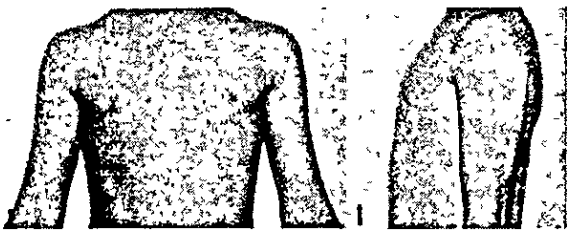
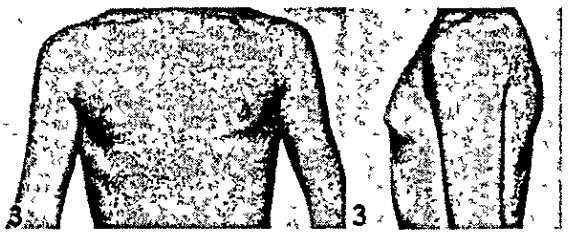
TANNER STAGES:
FEMALE BREAST DEVELOPMENT

The pictures on this page show different stages of development of the breasts. A girl passes through each of the five stages shown by these pictures. Please look at each of the pictures and read the sentences next to the picture. Then choose the picture closest to your stage of development and mark an **A** on the picture. Then choose the picture that is next closest to your stage of development and mark a **B** on the picture.

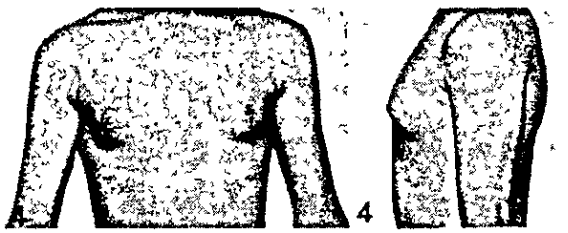


Stage 1
The nipple is raised a little in this stage. The rest of the breast is still flat.

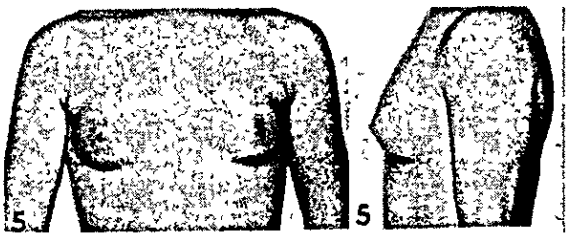
Stage 2
This is the breast bud stage. In this stage the nipple is little more raised. The breast is a small mound. The areola (darker, coloured middle part) is larger.



Stage 3
The areola and the breast are both larger than in stage 2, but the areola does not stick above the breast.



Stage 4
The areola and the nipple make up a mound that sticks up above the shape of the breast. (Note: this stage may not happen at all for some girls. Some girls develop from stage 3 to stage 5 with no stage 4).



Stage 5
This is the mature adult stage. The breasts are fully developed. Only the nipple stands out in this stage. The areola has flattened into the general shape of the breast.

Once you have completed the form, fold it and put it in the envelope provided and seal the envelope.

Your results are completely private and will be treated in complete confidence. No one will know who has filled out the form, as your name will not be on it.

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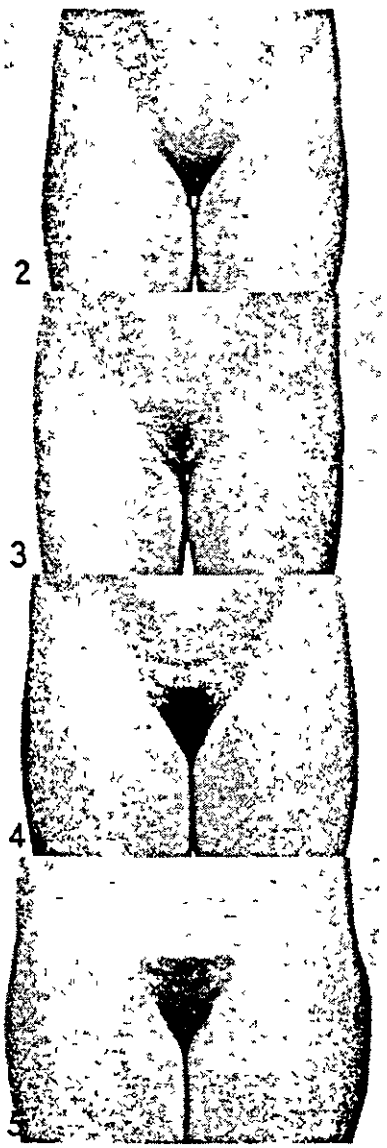
TANNER STAGES:
FEMALE PUBIC HAIR DEVELOPMENT

The pictures on this page show different stages of development of female pubic hair. A girl passes through each of the five stages shown by these pictures. Please look at each of the pictures and read the sentences next to the picture. Then choose the picture closest to your stage of development and mark an **A** on the picture. Then choose the picture that is next closest to your stage of development and mark a **B** on the picture.

Stage 1
(no picture)



Stage 1
There is no pubic hair at all.

Stage 2
There is a little soft hair. Most of the hair is along the slit or lips. This hair may be straight or a little curly.

Stage 3
The hair is darker in this stage. It is coarser and more curled. It has spread out and thinly covers a larger area.

Stage 4
The hair is now as dark as that of an adult woman. However, the area it covers is not as large as that of an adult woman. The hair has not spread out to touch the thighs.

Stage 5
The hair is now like that of an adult woman. It also covers the same area as that of an adult woman. The hair usually forms a triangular (∇) pattern as it spreads out to touch the thighs.

Once you have completed the form, fold it and put it in the envelope provided and seal the envelope.

Your results are completely private and will be treated in complete confidence. No one will know who has filled out the form, as your name will not be on it.