The effects of brisk walking on endurance fitness, lipoprotein metabolism and other risk factors for coronary heart disease

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THE EFFECTS OF BRISK WALKING ON ENDURANCE FITNESS, LIPOPROTEIN METABOLISM AND OTHER RISK FACTORS FOR CORONARY HEART DISEASE

by

David Stensel

A Doctoral Thesis

Submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy of the Loughborough University of Technology.

February 1993

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ABSTRACT

This thesis examines the potential of a one year programme of brisk walking to influence endurance fitness, lipoprotein metabolism and other risk factors for coronary heart disease (CHD) in previously sedentary, middle-aged men.

Seventy-two asymptomatic males were recruited into the study and randomly allocated on a two to one basis into either a walking group (n = 48) or a control group (n = 24). Walkers were asked to build up to an average of 45 minutes of brisk walking per day and maintain this level thereafter. Control subjects continued with their habitual lifestyle. Both groups undertook not to change their dietary habits.

Maximum oxygen uptake (VO₂max) was predicted at base line, three, six and 12 months from submaximal heart rate and oxygen uptake data during treadmill walking. Endurance fitness was assessed at similar intervals by measuring heart rate and blood lactate concentration during standardised submaximal treadmill walking. Serum concentrations of total cholesterol, high density lipoprotein cholesterol, apoproteins A-I and B, triglycerides and lipoprotein(a) were measured at each observation point. Additionally, plasma fibrinogen, resting arterial blood pressure and indices of pulmonary function were determined. Differences in the response of walkers and controls over time were examined using two way analysis of variance for repeated measures employing the 5% level of significance.

Adherence to the walking programme was high and 42 of the walkers (88%) remained in the study for the year completing an average of 28 ± 9 (mean ± SD) minutes of brisk walking each day (self-report). The response of the walkers over time was significantly different to that of the controls for predicted VO₂max and for heart rate and blood lactate concentration during standardised submaximal exercise indicating that brisk walking favourably influenced endurance fitness. No significant differences were observed between groups for any of the lipoprotein variables or other CHD risk factors monitored during the study.

It was concluded that brisk walking did not influence established CHD risk factors in this group of previously sedentary middle-aged men despite the evidence of improved endurance fitness.
ACKNOWLEDGEMENTS

I express my deepest gratitude to Dr Adrianne Hardman not only for her excellent help and advice but also for her close friendship throughout my studies. I could not have asked for a better supervisor. I thank Dr Katherine Brooke-Wavell and other contributors to this British Heart Foundation project including Professor Peter Jones, Dr Nick Norgan and Sara Herd for their comradeship while working with me on this project. I thank also my director of research, Professor Clyde Williams, for his inspiration and cheerful manner while I have been at Loughborough.

I am indebted to Mr David Vallance and Professor Tony Winder of the Department of Chemical Pathology at the Royal Free School of Medicine in London, for their advice and assistance with the lipid and lipoprotein measurements. I thank David in particular for his enthusiasm and friendship. I am grateful to Miss Jayshree Savania of the Department of Human Sciences at Loughborough University for carrying out the fibrinogen analysis and Dr Carol Jagger from the Department of Community Health at Leicester University for statistical advice.

Special thanks are due to the subjects for giving their time freely and for their high level of commitment throughout this long study.

Finally, I express my sincere thanks to the British Heart Foundation for their generous sponsorship of this work.
PRESENTATIONS AND PUBLICATIONS

Some of the findings included in this thesis have been presented/published elsewhere as follows:

Published communications:


Unpublished communications:

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**ABBREVIATIONS**

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<tr>
<td>ACSM</td>
<td>American College of Sports Medicine</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>apoprotein A-I</td>
</tr>
<tr>
<td>Apo B</td>
<td>apoprotein B</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>BL</td>
<td>base line</td>
</tr>
<tr>
<td>C</td>
<td>controls</td>
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<tr>
<td>CARDIA</td>
<td>Coronary Artery Risk Development in Young Adults</td>
</tr>
<tr>
<td>CHD</td>
<td>coronary heart disease</td>
</tr>
<tr>
<td>CV</td>
<td>coefficient of variation</td>
</tr>
<tr>
<td>FEV$_1$</td>
<td>forced expiratory volume in one second</td>
</tr>
<tr>
<td>FVC</td>
<td>forced vital capacity</td>
</tr>
<tr>
<td>HDL</td>
<td>high density lipoprotein</td>
</tr>
<tr>
<td>HDL-C</td>
<td>high density lipoprotein cholesterol</td>
</tr>
<tr>
<td>HMG CoA reductase</td>
<td>3-hydroxy-3-methylglutaryl coenzyme A reductase</td>
</tr>
<tr>
<td>HRmax</td>
<td>maximum heart rate</td>
</tr>
<tr>
<td>IDL</td>
<td>intermediate density lipoprotein</td>
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<tr>
<td>LCAT</td>
<td>lecithin: cholesterol acyltransferase</td>
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<tr>
<td>LDL</td>
<td>low density lipoprotein</td>
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<tr>
<td>LDL-C</td>
<td>low density lipoprotein cholesterol</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>lipoprotein(a)</td>
</tr>
<tr>
<td>PRE</td>
<td>perceived rate of exertion</td>
</tr>
<tr>
<td>R</td>
<td>respiratory exchange ratio</td>
</tr>
<tr>
<td>r</td>
<td>Pearson Product Moment correlation coefficient</td>
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<tr>
<td>rho</td>
<td>Spearman's rank order correlation coefficient</td>
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<tr>
<td>SEM</td>
<td>standard error of the mean</td>
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<tr>
<td>TC</td>
<td>total cholesterol</td>
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<tr>
<td>TG</td>
<td>triglyceride</td>
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<tr>
<td>VLDL</td>
<td>very low density lipoprotein</td>
</tr>
<tr>
<td>VLDL-C</td>
<td>very low density lipoprotein cholesterol</td>
</tr>
<tr>
<td>VE</td>
<td>minute ventilation rate</td>
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<tr>
<td>VE.$VO_2^{-1}$</td>
<td>ventilatory equivalent for oxygen</td>
</tr>
<tr>
<td>VO$_2$</td>
<td>volume of oxygen consumed</td>
</tr>
<tr>
<td>VO$_2$max</td>
<td>maximal oxygen uptake</td>
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1 INTRODUCTION

"Close to 100% of the existence of the hominid/homo species has been dominated by outdoor activities... now... we have in some 'privileged' societies ended up in an urbanized, highly technologic society dominated by a sedentary lifestyle" (Astrand, 1988). This relatively recent change in the habitual lifestyle of homo sapiens has been accompanied by associated changes in the occurrence of certain lifestyle diseases. In particular "... the incidence of coronary heart disease (CHD) increased relentlessly in technically developed countries during the first half of the 20th century and reached epidemic proportions in mid-century" (McGill, 1988). Furthermore, although the death rate from CHD is now falling in some westernised countries it is still a major cause of death. In the year 1990, for example, ischaemic heart disease accounted for 29% of all male deaths and 23% of all female deaths in England and Wales (Office of Population Censuses and Surveys, 1991).

The three major factors associated with increased risk of CHD are raised blood cholesterol concentration, hypertension and cigarette smoking but other factors also play a role. These include age, sex, obesity, diabetes and genetic predisposition (Kannel, 1988; Kuller and Orchard, 1988; Study Group; European Atherosclerosis Society, 1987). The possibility that a sedentary lifestyle might be implicated in the development of CHD was highlighted by the pioneering work of Morris and co-workers in the 1950s and 1960s (cited in Eichner, 1983 and Heyden and Fodor, 1988). These studies showed that men involved in physically active occupations had a lower incidence of CHD than their less active counterparts. Subsequently many other studies confirmed the relationship between physical activity and CHD. These included the investigations of Paffenbarger and colleagues on San Francisco longshoremen (1970) and on Harvard college alumni (1978) and the studies of Morris and co-workers on British civil servants (Morris et al. 1973; Morris et al. 1980).

Recently, many large and well designed studies have provided further evidence to support an independent and inverse relationship between physical activity and CHD (Donahue et al. 1988; Leon et al. 1987; Shaper and Wannamethee, 1991; Slattery et al. 1989; Morris et al. 1990). Moreover, Powell and colleagues (1987) in a review of 43 prospective studies on
physical activity and CHD concluded that: "The relative risk of physical inactivity is similar in magnitude to that of hypertension, hypercholesterolaemia and smoking".

Despite this mounting evidence, there is no definite proof of the 'exercise hypothesis'. As Blackburn and Jacobs (1988) state "a basic uncertainty is whether the effect (of exercise) is due to environment [activity] or constitution [genes]... Fitness, with its strong constitutional component may... determine both who will be fit and who will be protected from clinical CHD". Although the notion that fitness has a strong constitutional component could be challenged, on the grounds that training can profoundly influence an individual's capacity for exercise, it is evident that an intervention study seeking to determine if there is a strong and independent effect of physical activity on CHD rates in previously sedentary subjects is unlikely to be conducted. Not only would it be immensely expensive but it would also be extremely difficult to control and require a huge logistical effort (Despres, 1989; Tunstall Pedoe, 1990). Notwithstanding this fact, however, many authorities believe that it is prudent to regard exercise at least as a minor and if not a major risk factor for CHD due to the strength of the epidemiological evidence (Gordon and Scott, 1991; Sadlo and Wenger, 1990).

The means by which exercise might protect against CHD is the subject of debate but is thought by many to include an effect on lipoprotein metabolism. Although the evidence suggesting that exercise can lower total cholesterol or low density lipoprotein cholesterol (LDL-C) is equivocal, many studies have shown that exercise is an effective means of elevating high density lipoprotein cholesterol (HDL-C) (see Haskell, 1986). High density lipoprotein cholesterol is negatively associated with heart disease and is increasingly becoming accepted as a strong and independent risk factor for CHD (Rifkind, 1990). Intervention trials using diet and/or drug treatments have now provided strong evidence to demonstrate that lowering serum cholesterol reduces mortality rates from CHD (see Rossouw and Rifkind, 1990; Ball and Mann, 1988). Moreover, results from the Helsinki Heart Study suggest that interventions which increase HDL-C concentration are also of benefit in reducing CHD mortality (Frick et al. 1987). If exercise is an effective means of reducing total cholesterol or LDL-C and/or increasing HDL-C it would seem likely that it should lead to a reduced incidence of CHD.
The mode of exercise most often associated with increased concentrations of HDL-C is endurance training involving activities such as running, swimming and cycling (Wood et al. 1984; Fang et al. 1988). Studies attempting to relate improvements in endurance fitness with increased concentrations of HDL-C have not always been successful however (Allison et al. 1981; Brownell et al. 1982; Haskell et al. 1980). One reason for this may be that the traditional physiological index used to appraise changes in endurance fitness, the maximal oxygen uptake (VO\textsubscript{2}max), is a relatively insensitive measure in this respect. A high VO\textsubscript{2}max is a prerequisite for elite endurance performance because of the strong linear relationship between oxygen uptake and speed of running, swimming or cycling. However, VO\textsubscript{2}max is to a large extent genetically predetermined (Klissouras et al. 1973) and training programmes have been found to improve endurance performance dramatically with little or no effect on VO\textsubscript{2}max values (Williams and Nute, 1982; Lortie et al. 1984).

The insensitivity of VO\textsubscript{2}max as a marker for changes in endurance fitness may relate to the fact that improved endurance (the ability to maintain a given exercise pace or intensity for longer) is largely the result of peripheral rather than central factors i.e. an improvement in the oxidative capacity of skeletal muscle (Gollnick et al. 1973; Davies et al. 1981; Hardman and Williams, 1983). An enhanced oxidative capacity of skeletal muscle facilitates endurance performance by reducing the anaerobic contribution to energy provision at a given exercise intensity thus delaying fatigue (Matoba and Gollnick, 1984). A reproducible means of indirectly assessing changes in the oxidative capacity of skeletal muscle may be obtained through the measurement of blood lactate concentration during submaximal exercise. Running studies have shown that changes in blood lactate concentration during submaximal exercise provide a better indication of improvements in endurance fitness than VO\textsubscript{2}max per se (Sjodin and Svedenhag, 1985; Williams and Nute, 1986). There is a need then to re-examine the relationship between changes in endurance fitness and HDL-C using a more sensitive index of training adaptations than the traditional VO\textsubscript{2}max measurement.

Aside from its potential to modify cholesterol metabolism there is also evidence that exercise may be effective as a means of ameliorating another of the major risk factors for CHD, hypertension (Tipton, 1991). Moreover, there is limited evidence that exercise has a beneficial influence on additional risk factors which are linked to the development of heart disease including plasma
fibrinogen concentration (Ernst, 1990), lipoprotein(a) concentration (Hellsten et al. 1989) and lung function (Shaper and Wannamethee, 1991). The above three factors have recently become more prominent as markers for CHD risk and thus an examination of the potential of exercise to modify them is justified.

Assuming that physical activity does have the potential to modify CHD risk its ability to make an effective contribution towards the reduction of CHD mortality in the U.K. depends on at least two other factors. The first of these factors is the current level of physical activity amongst the U.K. population. If participation rates in physical activity are already high then efforts to reduce CHD risk would be better focused on other areas. This does not appear to be the case, however. Until recently there was only limited evidence regarding participation rates in physical activity amongst the U.K. population. This came from the General Household Survey and the Welsh Heart Study (cited in Jacobson et al. 1991) both of which indicated that physical activity levels amongst the general population were low. This year, however, the results of the National Fitness Survey have been published. These provide a much more detailed examination of physical activity patterns in the U.K.

The National Fitness Survey was conducted in England and examined 3,949 men and women aged between 16 and 74 years old. Questionnaires were used to assess the mode, duration, frequency and intensity of exercise completed by each individual over a four week period. The results of this survey indicated that only 14% of men and 4% of women took part in vigorous physical activity three times per week for at least 20 minutes on each occasion i.e. "... the level of activity often identified as valuable for building stamina and offering protection against CHD" (The Sports Council and the Health Education Authority, 1992). Vigorous activities were defined as those requiring an energy expenditure of above 7.5 kilocalories per minute and were equated with an exercise intensity of above 60% of VO$_{2}$max. The survey also found that there was a decline in physical activity with increasing age, especially vigorous activity.

A second important criterion for any type of intervention which seeks to improve the primary prevention of CHD is that it is socially acceptable to the population which it is aimed at. In this respect, it is of interest that the National Fitness Survey found that in each age group, over 50% of the men and women interviewed reported that they had walked continuously for at least a
mile in the week prior to interview. The survey concluded that "...the high prevalence of walking among men and women of all ages... suggests that there is scope for increasing activity levels by encouraging those who do walk to do so more frequently, for longer distances and at a faster pace". It should also be emphasised that there is a lower adherence to, and higher incidence of injuries during, high intensity exercise programmes, such as running, than with less vigorous forms of exercise such as walking (Martin and Dubbert, 1982; Seals et al. 1984). An appropriate form of exercise then in a population based strategy for the primary prevention of CHD might be brisk walking since this is a form of exercise which is likely to be acceptable to a majority of people.

It has been demonstrated that brisk walking can serve as an adequate aerobic training stimulus for middle-aged men and women (see Porcari et al. 1989). In addition Cook and co-workers (1986) found a relationship between the reported number of miles walked per day and HDL2-C concentration in active postal carriers. Moreover, there is epidemiological evidence to support a beneficial effect of walking exercise on all-cause mortality (Paffenbarger et al. 1986) and Morris and colleagues (1990) have shown that men claiming that their regular speed of walking is fast (>4.0 mph) suffer a lower incidence of CHD than others.

Recently a study from this laboratory examined the influence of brisk walking on lipoprotein metabolism in a group of previously sedentary middle-aged women and found a 27% increase in HDL-C concentration over a one year period (Hardman et al. 1989a). To the author's knowledge, however, there have been no controlled, randomised studies examining the effects of a prolonged (one year) programme of brisk walking on blood lipids and other CHD risk factors in previously sedentary men. Yet risk factor reduction in men should have a greater impact on the total number of deaths attributable to CHD than changes in women because there is a higher incidence of CHD in men than in women.

The purpose of the present study then was to examine the influence of a one year programme of brisk walking on endurance fitness, lipoprotein metabolism and other CHD risk factors in a group of previously sedentary, asymptomatic, middle-aged men. In particular this study sought firstly to determine the effect of brisk walking on endurance fitness. This was assessed by monitoring
changes in blood lactate concentration during submaximal exercise which are thought to provide a more sensitive index of changes in endurance fitness than the traditionally used VO₂max measurement. Secondly, this study sought to examine the potential of brisk walking to modify lipoprotein metabolism and in particular to increase HDL-C concentration. Finally, the effect of brisk walking on other markers for cardiovascular disease including blood pressure, pulmonary function and plasma fibrinogen concentration was assessed. Thus, this study represents a thorough examination of the potential of brisk walking to modify a number of factors related to CHD risk.
2 REVIEW OF LITERATURE

2.1 Overview

This review begins by discussing the epidemiological evidence linking physical activity and CHD. In the next Section the different types of lipids and lipoproteins are defined, their role in lipoprotein metabolism is explained and their possible relationships with CHD are discussed. After this there is a short Section on the potential sources of error inherent in lipid and lipoprotein measurement including biological and analytical variation. The interaction between exercise and lipoproteins is then reviewed and possible mechanisms which may explain this interaction are examined. Following this the relationship between exercise and other selected risk factors for CHD including hypertension, elevated plasma fibrinogen concentration and impaired pulmonary function is addressed. In the final Section the cardiovascular and metabolic adaptations to endurance exercise are investigated. This Section includes a discussion on the use of measurements of blood lactate concentration in assessing improvements in endurance fitness. Also included is an assessment of the effectiveness of walking exercise as a means of enhancing endurance fitness.
2.2 Exercise and coronary heart disease: epidemiological evidence

Evidence to suggest that there may be a link between exercise and CHD first emerged in the middle of this century. Logan (1952) collected mortality data in England from 1931 to 1951. This data revealed an enormous increase in coronary deaths and a remarkable difference among the social classes. Coronary deaths were most common in professional men and least common in unskilled workers. These findings implied that individuals engaged in sedentary occupations were predisposed to an increased risk of CHD. The “exercise hypothesis” was born.

The following year Morris and colleagues (1953) presented data on a group of 31,000 London Transport workers many of whom were either bus drivers or bus conductors. This data showed that rates of myocardial infarction and sudden death were significantly lower among bus conductors, whose occupations required more physical activity, than bus drivers. This study was subsequently challenged on the basis that driver recruits tended to be fatter than conductor recruits, suggesting that different men chose different jobs. However, Morris (1959) subsequently showed that all drivers, including those who were thin, had a higher incidence of sudden death than the corresponding conductors. Morris (1959) concluded that “… physique is not the crucial factor in producing the occupational differences reported”.

The pioneering work of Morris and colleagues stimulated great interest in the relationship between exercise and CHD and their findings were confirmed in many subsequent studies. The populations under observation in these studies were diverse and included white males employed by the United States railroad industry (Taylor et al. 1962), black and white men from Evans County, Georgia (McDonough et al. 1965), San Francisco Longshoremen (Paffenbarger et al. 1970) and Jewish men and women living in kibbutzim in Israel (Brunner et al. 1974) to name just a few. These studies have been reviewed by many authors (i.e. Eichner, 1983; Heyden and Fodor, 1988; Murray et al. 1989; Sadlo and Wenger, 1990) but probably the most systematic review is that of Powell and colleagues (1987). These authors collected 121 articles representing at least 54 studies examining physical activity and CHD. Of these, 43 met up to their selection criteria and were included in their review. These 43 studies provided information primarily about
North American and European working-aged men. Powell and colleagues (1987) found that "the relative risk of CHD associated with inactivity varies among studies but generally ranges from 1.5 to 2.4, with a median of about 1.9... (Moreover), better studies tended to report higher relative risks". These findings have been reaffirmed recently in a meta-analysis performed by Berlin and Colditz (1990) and they are significant because, as noted by Powell and colleagues (1987), they suggest that the relative risk of inactivity is equivalent in size to that of hypertension, hypercholesterolaemia and smoking.

Sadlo and Wenger (1990) have emphasised that one of the shortcomings of many of the earlier studies was that they focused more on the measurement of coronary events than on the measurement of physical activity. Moreover, Powell and colleagues (1987) point out that the operational definition of physical activity is crucial to the value of epidemiological studies and the unsatisfactory physical activity measures used in many of the earlier investigations meant that comparison between these studies was difficult. The last two decades have seen a shift in the emphasis of epidemiological studies from occupational activity to leisure-time activity. Moreover, attempts have been made to improve the way in which physical activity is assessed and quantified. As a result the evidence linking exercise with the development of CHD has been strengthened. However, there have been some conflicting findings regarding the amount and intensity of physical activity required to provide protection from CHD.

In Britain, Morris and colleagues (Morris et al. 1973) followed up their earlier studies by examining leisure-time exercise in 16,882 middle-aged, male civil servants. Leisure-time physical activity was assessed from detailed individual records of all activity over a two day period. The results revealed that "in men recording vigorous exercise the relative risk of developing coronary disease was about a third that in comparable men who did not... Lighter exercise, and provisional estimates of overall activity, showed no such advantage" (Morris et al. 1973). Vigorous was defined here as exercise likely to result in peaks of energy output of 7.5 kcal. per minute and an oxygen uptake above 1.5 l.min⁻¹. This included such activities as active recreations (e.g. swimming), "keep fit" exercises, heavy work (e.g. digging) and getting about quickly.
In their most recent study of leisure-time activity in 9,376 male civil servants, Morris and colleagues (1990) have confirmed their earlier findings. Men reporting vigorous physical activity experienced less than half the non-fatal and fatal CHD of other men while, once again, non-vigorous exercise or high totals of physical activity per se offered no protection. There were, however, some interesting differences between this study and the previous one. In the more recent study, for example, recreational heavy work, which previously had been weakly associated with protection from CHD, was not beneficial. Morris and colleagues (1990) hypothesised that this was due to the fact that recreational heavy work is often periodic (i.e. seasonal) or occasional. Moreover, the nature of heavy work, which often "... entails discontinuous lifting and carrying, pushing and pulling of heavy objects" is very different from the "... predominantly dynamic, rhythmic contraction of large skeletal muscles" which occurs in such activities as swimming, walking, running and cycling. The implication here is that some adaptation associated specifically with endurance exercise, i.e. the type of exercise likely to elicit a cardiorespiratory training effect, is responsible for the protection which is gained from CHD.

Another interesting finding from Morris and colleagues' (1990) more recent study was that amongst the older men (i.e. those aged 55 to 64 at entry) there was evidence of a dose response relationship between vigorous aerobic exercise and CHD. Thus, when the men were divided into four groups according to the frequency and intensity of exercise, group one who reported the most frequent/intense vigorous aerobic exercise had the lowest incidence of CHD. However, the men in group two and to a lesser extent group three, where exercise was either not so frequent or not so intense i.e. "fairly brisk walking" instead of "fast walking", still had a lower incidence of CHD than the men in group four who reported no vigorous aerobic exercise. This trend was not evident in the younger men (i.e. those aged 45 to 54 at entry) where only a threshold effect was seen. However, it is important to note that even in the older men it was still only vigorous exercise which was protective even if there was less of it or the intensity was slightly reduced. Thus, it may be supposed that with advancing age the absolute exercise intensity required to provide protection from CHD may be lower providing that, in relative terms, the exercise is still vigorous.
While Morris and colleagues pursued their investigations in Britain, Paffenbarger and co-workers followed their examination of San Francisco longshoremen with a study of physical activity and all-cause mortality in 16,936 Harvard college alumni (Paffenbarger et al. 1978; Paffenbarger et al. 1984; Paffenbarger et al. 1986). Questionnaires were used to assess the amount of time spent each week in activities such as walking, stair climbing and light and vigorous sports play. The weekly energy expenditure resulting from these activities was then estimated and used as an index of physical activity. The results were very interesting since they showed that death rates (the major cause of death was cardiovascular disease) declined steadily as energy expenditure increased from less than 500 to 3500 kcal. per week, beyond which there was a slight increase in rates (Figure 2.1).

![Figure 2.1](image.png)

Figure 2.1 The relationship between physical activity and mortality rates in Harvard college alumni (Paffenbarger et al. 1986). Redrawn from Murray et al. (1989).

Death rates were between one quarter and one third lower among alumni expending 2000 or more kcal. during exercise each week than among the less active men. This relationship was independent of such factors as hypertension and cigarette smoking. One other important finding from the Harvard alumni
study was that only those ex-varsity athletes who maintained a high physical activity index were at a reduced risk of death while sedentary students who subsequently became physically active acquired low risk (Paffenbarger et al. 1984). Morris and colleagues (1990) made similar observations in their study of civil servants i.e. a history of vigorous sports in the past was not protective. Together these findings argue against a self-selection effect.

The studies of British civil servants (Morris et al. 1973; Morris et al. 1980; Morris et al. 1990) and Harvard college alumni (Paffenbarger et al. 1978; Paffenbarger et al. 1986) provide strong support for the hypothesis that exercise and CHD are inversely related. However, since only "vigorous" aerobic exercise was associated with a reduced incidence of CHD in the studies of Morris and colleagues the implication is that exercise must provide a training effect on the cardiovascular system in order to be protective. Paffenbarger's findings, on the other hand, suggest that it is the overall level of energy expenditure which is important regardless of exercise intensity.

It is difficult to reconcile the above findings although they may be due in part to differences in study design, as discussed below. It is important to recognise, however, that energy expenditure was not determined directly in these studies but was estimated with the aid of published average values and therefore some inaccuracy in measurement was inevitable. In the Harvard college alumni study (Paffenbarger et al. 1978), for example, the values of Passmore and Durnin (1955) were used. Thus, sporting activity was graded as light, strenuous or a combination of the two and assigned an energy expenditure of either 5, 10 or 7.5 kcal. per minute respectively. Weekly energy output (kcal. week\(^{-1}\)) was then calculated from records of the time spent in these activities. Yet this fails to take into account the fact that the energy expenditure of any given activity will vary greatly depending on the intensity of that activity. For example, a 65 kg man running at a speed of 11 minutes and 30 seconds per mile will expend less than half the energy (8.8 kcal.min\(^{-1}\)) of a man whose mass is similar but whose running speed is 5 minutes and 30 seconds per mile (18.8 kcal. per minute) (McArdle et al. 1981). Moreover, body weight was not considered in the estimations of energy expenditure. Again this is important since, to take another example, a 50 kg man running at a speed of nine minutes per mile will expend approximately half the energy of a 98 kg man running at the same speed (9.7 kcal.min\(^{-1}\) versus 18.9 kcal.min\(^{-1}\) respectively) (McArdle et al. 1981). Thus, it is possible that many individuals
were placed into inappropriate activity (energy expenditure) bands. Similarly, there may have been some misclassification in the study of Morris and colleagues (1973) where activities such as swimming and dancing were listed as vigorous although they would not necessarily elicit peaks in energy expenditure of 7.5 kcal per minute in all individuals.

The disparity between Paffenbarger's findings and those of Morris and colleagues could be due to the way in which the former's physical activity index was calculated. This index was a measure of the total energy expended in all assessed activities and thus is insensitive to the different physiological effects of light and strenuous activities. Furthermore, no attempt was made to assess the speed of walking or stair climbing in the Harvard alumni study though again the physiological effects of these activities will vary depending on the speed/intensity at which they are performed. Therefore, it is possible that the majority of the relationship between total energy expenditure and heart attack risk was due to the energy expended in the more vigorous activities e.g. strenuous sports and relatively brisk walking/stair climbing. In other words, the type of activities likely to elicit a cardiovascular training effect and therefore confer some protection from CHD. This suggestion is supported by the finding of Paffenbarger and colleagues (1978) that "... at any given level of energy expenditure, the risk of heart attack tends to be lower with strenuous sports than with more casual activities".

Aside from the studies of Morris and co-workers and Paffenbarger and colleagues a few other investigations have also grouped individuals according to the type and quantity of physical activity performed. One such study is the Multiple Risk Factor Intervention Trial (Leon et al. 1987). This study examined the relationship between leisure-time physical activity and CHD and overall mortality in a high risk group of North American middle-aged men. Physical activity was assessed using the Minnesota Leisure-Time Physical Activity Questionnaire and subjects were divided into tertiles (low, moderate and high) based on the amount of time and energy expended in physical activity. The low activity group averaged about 107 minutes of leisure-time physical activity per week at base line and expended about 500 kcal. compared with about 332 minutes and 1500 kcal. per week for the moderately active group and 935 minutes and 4400 kcal. per week for the most active group. During the seven years of follow-up there were only 63% as many fatal CHD events and sudden deaths and only 70% as many total deaths in the
moderate activity group compared to the low activity group. Mortality rates in the high activity group were similar to those in the moderate activity group.

As with the study of Harvard college alumni, the findings of the Multiple Risk Factor Intervention Trial appear to conflict with those of the British civil servants studied by Morris and colleagues. However, an examination of the activity gradings in the Multiple Risk Factor Intervention Trial reveals that they are dissimilar to the gradings used by Morris and colleagues. For example, walking for pleasure and bicycling were graded as light by Leon and co-workers (1987) and gardening, yard work, home repairs, dancing, swimming and home exercise were graded as moderate. Morris and colleagues (1973), however, graded brisk walking, cycling, dancing, swimming, keep fit exercise and some types of gardening and do-it-yourself work as vigorous. It is possible then, that much of the exercise performed by the moderate activity group in the Multiple Risk Factor Intervention Trial would have been classified as "vigorous" by Morris and colleagues. If this is the case then the findings of these studies are not as contradictory as they at first appear.

The U.S. Railroad Study (Slattery et al. 1989) also examined the relationship between the energy expended in leisure-time physical activity and death from CHD. The participants in this study were 3,043 white males. As in the Multiple Risk Factor Intervention Trial, leisure-time physical activity was assessed using the Minnesota Leisure-Time Physical Activity Questionnaire. The results of this study showed that there was a 30% to 40% greater risk of dying from CHD or from all-causes in sedentary individuals compared with those who expended over 1000 kcal. per week in leisure-time physical activity. There was little further reduction in mortality risk, however, beyond 1000 kcal. per week of activity. These results are similar to those of the Multiple Risk Factor Intervention Trial. Once again, however, some of the activities graded as moderate in the U.S. Railroad Study would have been graded as vigorous in the studies of Morris and colleagues.

One other recent study worthy of mention is the British Regional Heart Study (Shaper and Wannamethee, 1991). This study examined the relationship between leisure-time physical activity and ischaemic heart disease in middle-aged men. A total of 7,735 men who were representative of the socioeconomic distribution of men in Great Britain were included in the study. Some of these men had pre-existing ischaemic heart disease. A physical activity score was
calculated for each individual based on the amount of time they spent engaged in regular walking, cycling, recreational activity and sporting activity. For the recreational and sporting classifications the type of activity was also taken into account thus giving a relative measure of energy expenditure. The men were grouped into six broad categories based on their total scores. The category headings were: inactive, occasional, light, moderate, moderately vigorous and vigorous. The follow up period was eight years and during this time the men who were inactive had a significantly higher heart attack rate than all other men. The lowest heart attack rates were seen in the moderate and moderately vigorous categories and men in these categories had a 50% reduction in heart attack risk compared to men in the inactive group. Furthermore, when all men who reported doing sporting exercise at least once a month were excluded from the analysis, a strong inverse relationship between physical activity and heart attack risk remained in men without pre-existing ischaemic heart disease. The authors concluded that "the overall level of physical activity is an important independent protective factor in ischaemic heart disease and that vigorous (sporting) exercise, although beneficial in its own right, is not essential in order to obtain such an effect" (Shaper and Wannamethee, 1991).

Although the British Regional Heart Study provides further evidence to support the link between exercise and CHD there are some shortcomings in the way in which this study categorised physical activity. A major criticism is that this study equated sporting activity with vigorous activity. Thus, vigorous was defined as "very frequent sporting exercise or frequent sporting exercise plus other recreational activities". As a result of this, activities such as golf and sailing were graded as vigorous exercise. Although during certain periods sailing can be "hard physical work" when performed by national competitors (Shephard, 1990) it is unlikely that many of the men in the British Regional Heart Study would have performed this activity at an intensity sufficient to be described as vigorous. Furthermore, it is doubtful that golf would qualify as vigorous at any level of play although it may be an attractive activity for many overweight men who feel that they should do some exercise.

In addition to the above, no reported attempt was made at measuring the speed of walking or cycling, two of the major activities assessed in the British Regional Heart Study. Only the amount of time spent in these activities was taken into account. As a result of this many of the men may have been given
inaccurate physical activity ratings. For example, a man walking at a speed of 4.5 miles per hour for 40 minutes would have been given a similar physical activity score to that of a man walking 3 miles per hour for 40 minutes, yet the distance walked would be 3 miles in the first instance and only 2 miles in the second. Thus, the second man would expend only two-thirds of the energy of the first man provided that the body mass of the two men did not differ. Moreover, although an attempt was made to validate the physical activity score by correlating it with measures of physical fitness, the fitness measures employed, i.e. resting heart rate and forced expiratory volume in one second, are of doubtful validity and are not widely used (Brooks and Fahey, 1984; McArdle et al. 1981).

From the preceding discussion it is clear that although more has been learned about the relationship between physical activity and CHD there are still many issues to be resolved. One major issue is the apparent difference in the findings of Morris and colleagues and those of others i.e. whether it is physical activity per se or physical fitness which protects from CHD. It has already been noted that one explanation for the inconsistencies between studies may be the differential grading of various physical activities in the studies. Another possibility is that the differences are related to the nature of the populations under observation. For example, Morris and colleagues (1990) state that the participants in their most recent study were "... probably more healthy and fit than average because they are a working population and because we excluded men with a clinical history of coronary heart disease; they were more active too than the national population of their age and social class". In contrast to this the population studied by Leon and colleagues (1987) in the Multiple Risk Factor Intervention Trial was a high risk population and some of the participants in the British Regional Heart Study (Shaper and Wannamethee, 1991) had pre-existing ischaemic heart disease. On this basis it may be that the participants in the studies of Morris and colleagues were fitter than those in many of the other studies and therefore they required a greater exercise stimulus to gain additional protection from CHD.

The recent trend to assess the relationship between physical fitness and CHD may help to resolve some of the uncertainties regarding the interrelationships between physical fitness, physical activity and CHD. An inverse relationship between physical fitness and CHD has now been shown by many studies. These include the Dallas Aerobics Centre Study (Blair et al. 1989), the
Belgian Physical Fitness Study (Sobolski et al. 1987) the Lipid Research Clinics Mortality Follow-up Study (Ekelund et al. 1988), the U.S. Railroad Study (Slattery and Jacobs, 1988) and studies carried out in Los Angeles (Peters et al. 1983) and Norway (Eriksson, 1986). In three of these studies (Blair et al. 1989; Ekelund et al. 1988; Sobolski et al. 1987) the relative risks of CHD, obtained when comparing those in the top and bottom fitness groups, were much higher than the relative risks of physical inactivity found in large scale epidemiological studies.

Despite this evidence of a link between physical fitness and CHD there are still problems to be resolved. Of crucial importance is the way in which physical fitness is defined and measured. In the studies mentioned above fitness was defined in absolute terms. Blair and colleagues (1989), for example, used total treadmill time on a maximal exercise test as their measure of fitness and Sobolski and co-workers (1987) used the relative physical working capacity to indicate fitness i.e. the work load attained at a heart rate of 150 beats.min$^{-1}$, divided by body weight. The problem with these definitions is that they are influenced by inherited characteristics to an important degree. In Blair and colleagues' study, for example, treadmill time was highly correlated with VO$_2$max and the latter variable is known to have a strong genetic component (Kliissouras et al. 1973). Similarly, because of the strong relationship between work rate and oxygen uptake, VO$_2$max would have been an important determinant of fitness as measured in the study of Sobolski and co-workers (1987).

Because of the inherent genetic influence when fitness is defined in absolute terms it is difficult to quantify the contribution which physical activity has made to CHD protection. In some cases it does not appear to be that great. In the Belgian Physical Fitness Study, for instance, correlations between leisure-time physical activity and fitness and between occupational physical activity and fitness were 0.10 and 0.07 respectively. Given that physical activity should have some influence on physical fitness this suggests that either the measures of physical activity or those of physical fitness were inappropriate. In future studies it would be useful if fitness could be defined and measured in relative terms e.g. by measuring the heart rate or blood lactate concentration at a given relative exercise intensity. If the definition and measurement of physical fitness can be improved in future studies and a relationship between physical fitness and CHD is still found to exist, then this will give greater credibility to
the assertion that physical activity rather than constitutional factors affects physical fitness and therefore CHD. Despite their limitations, however, the studies which have examined physical fitness and CHD have been of value since they have confirmed that these two variables are related. Moreover, since a dose response relationship between physical fitness and CHD has been demonstrated in some studies (Blair et al. 1989; Ekelund et al. 1988; Sobolski et al. 1987) and since Morris and colleagues (1990) found a dose response relationship between the frequency of vigorous aerobic exercise and CHD incidence in their study (for men aged 55 to 64 at entry), it may be postulated that the greater the effect which physical activity has on physical fitness, the greater will be the benefit in terms of protection from CHD.

Just as improvements in the measurement of physical fitness are required, so are improvements in the way in which physical activity is measured. These may help to reconcile some of the conflicting findings from previous epidemiological studies. The predominant tool used to measure physical activity by epidemiologists, the questionnaire, has inherent limitations. There will always be inaccuracies in recall data and this will reduce the precision with which physical activity can be quantified. To give an example, in the Multiple Risk Factor Intervention Trial the relationships between leisure-time physical activity at base line and at the first, fourth and sixth annual visits were all weak ($r = 0.38$, 0.31 and 0.30 respectively) (Leon et al. 1987). Slattery and colleagues (1989) also reported poor relationships between physical activity scores on the first and second assessment in the U.S. Railroad Study. This disparity probably reflects both true changes in physical activity and also inaccurate reporting.

Despite these problems it is difficult to see an alternative to the use of questionnaires for assessing physical activity in large scale epidemiological studies. However, a greater degree of consistency between questionnaires as regards the grading of physical activity would be an attainable goal. Subjects could also be asked to keep a record of activity over some future period as an adjunct to the questionnaires used to measure previous exercise habits. Attempts could be made to assess the speed and intensity of activities such as walking, cycling and swimming, at least in a subgroup of study participants. Measurements of distance and time could be used to get an estimation of speed and portable pulse rate monitors could be employed to measure the heart rate of subjects over one or a number of days. Another means of
validating intensity scores would be to use Borg's scale of perceived exertion and to ask questions regarding the symptoms associated with exercise, i.e. heavy breathing and sweating, as suggested by Morris (personal communication). If future studies can improve on the accuracy with which physical activity is measured and classified this should allow for a more confident identification of the types of exercise most closely associated with reduced CHD risk. This in turn would have important consequences for the promotion of various forms of physical activity.

In conclusion it may be stated that although there is still much to be learned, the relationship between exercise and CHD is now firmly established. It appears that the most beneficial type of activities for reducing CHD risk are those which are endurance based. That is, activities which are sustained, rhythmic and aerobic in nature such as walking, jogging, swimming and cycling and which have an important effect on the heart and cardiovascular system. As to the amount of exercise required to reduce CHD risk this will obviously vary depending on an individual's age and fitness level as well as other factors. Whether vigorous exercise is essential or not remains contentious but evidence certainly suggests that this form of exercise is the most beneficial. This is supported by the fact that even in studies where overall energy expenditure is found to be protective, energy expended in vigorous exercise is usually associated with a lower risk than energy expended in non-vigorous exercise (Paffenbarger et al. 1978; Slattery et al. 1989). It should be noted, however, that the energy expenditure considered to represent vigorous exercise by Morris and colleagues (i.e. 7.5 kcal.min\(^{-1}\)) is still somewhat lower than the highest levels of energy expenditure which are possible during exercise. Moreover, Morris and colleagues (1980) themselves note that the men which they studied were no athletes. Therefore, vigorous exercise according to the above definition still represents an attainable goal for most individuals.

The mechanisms by which exercise might lead to a reduction in CHD risk are not well understood. Some of the influence of exercise on CHD may be mediated through the effect which exercise has on other CHD risk factors such as lipoprotein metabolism, hypertension and obesity. However, in most of the epidemiological studies discussed above the relationship between exercise and CHD remained significant (though it was sometimes attenuated) even when other risk factors were controlled for. This suggests that at least part of
the reduction in risk associated with physical activity results from a direct influence of that activity. In the following Sections of this review, some of the plausible mechanisms by which exercise may directly or indirectly reduce the risk of CHD are examined.
2.3 Lipids, lipoprotein metabolism and coronary heart disease

2.3.1 Lipids

Lipids, which account for approximately 40 per cent of the body's organic matter, are molecules composed of predominantly carbon and hydrogen atoms. These atoms are joined by electrically neutral covalent bonds which means that the resulting lipid molecules are nonpolar and thus insoluble in water since they have no electrical affinity for water molecules. It is this insolubility in water that characterises lipids. There are three major subclasses of lipids: triglycerides (also known as triacylglycerols), phospholipids and steroids. In addition, fatty acids compose another class of lipids which are either incorporated into triglycerides and phospholipids or exist simply as "free" molecules (Vander, Sherman and Luciano, 1990).

Each of the lipid subclasses has a distinct role to play in the maintenance of normal body function. Phospholipids, for example, form a major component of cell membranes while steroids are precursors of bile acids, used in lipid digestion, and steroid hormones, such as the sex hormones, in addition to their role as constituents of cell membranes. One very important steroid is cholesterol which has received considerable attention in the scientific world recently because of its atherogenic properties. The most abundant source of lipid in the body, however, occurs in the form of triglycerides which are often referred to simply as fat. Triglycerides are highly concentrated stores of metabolic energy which serve predominantly as energy substrates. They are formed by the joining together of glycerol, a three-carbon carbohydrate, with three fatty acid molecules, a process termed esterification. This process is reversible i.e. the fatty acid molecules may be cleaved from their glycerol backbone resulting in triglyceride dissolution, this is known as lipolysis or hydrolysis. Digestion of dietary fats then involves the lipolysis of triglycerides which then require re-esterification if the consumed lipids are to be stored. Utilisation of the stored triglycerides i.e. for energy metabolism, will require a further lipolysis (Brooks and Fahey, 1984; Tortora and Anagnostakos, 1981).
2.3.2 Lipoproteins

As mentioned previously most lipids are non-polar and thus insoluble in water. Therefore, in order that they can be transported around the body to perform their required functions they must combine with apolipoproteins (also termed apoproteins) to form lipoproteins which are soluble in plasma due to their complex arrangement. Thus, lipoproteins are macromolecular complexes composed of various lipids (cholesterol, triglycerides and phospholipids) and protein. They exist in a state of dynamic equilibrium among themselves with a continuous exchange of lipid and protein between macromolecules. Furthermore, all lipoproteins share a common structural make up consisting of a hydrophobic core of lipids (triglycerides and cholesterol esters) and a hydrophilic shell of polar lipids (phospholipids and free cholesterol) and apoproteins (Stryer, 1988). Nevertheless, lipoproteins can be divided into five basic categories according to density (determined by ultra-centrifugation) as described by Assmann (1982).

Chylomicrons (density<0.95 g.ml⁻¹) are formed in the intestinal mucosa and transport exogenous (dietary) triglycerides. They are composed of 98-99.5% lipid and 0.5-2% protein (primarily apoproteins A-I and C). After entering the systemic circulation chylomicrons have a half-life of only a few minutes, being degraded to core remnants. Thus, in the absence of metabolic disease, chylomicrons are found only in post-prandial serum and not in fasting serum.

Very low density lipoproteins (VLDL) (density<1.006 g.ml⁻¹) are formed in the liver and transport the bulk of endogenous triglycerides. They consist of 85-90% lipid and 10-15% protein (primarily apoproteins B-100, C and E).

Intermediate density lipoproteins (IDL) (density = 1.006-1.019 g.ml⁻¹) are found only in very low concentrations in normal individuals and are thought to be metabolic products of VLDL which then become precursors for LDL. Apoproteins B-100 and E are present in IDL.

Low density lipoproteins (LDL) (density = 1.019-1.063 g.ml⁻¹) arise as metabolic products of VLDL and are responsible for transporting the bulk of cholesterol in the blood. They contain approximately 75% lipid and 25% protein (apoprotein B-100).
High density lipoproteins (HDL) (density = 1.063-1.210 g.ml⁻¹) function primarily in cholesterol esterification and exchange reactions within the plasma. Thus, HDL receive cholesterol from other lipoproteins and extrahepatic cells, modify it and then return it for transport back to the liver. High density lipoproteins also serve as a 'reservoir' for apoproteins during lipoprotein metabolism (Brewer et al. 1988). They contain approximately 50% lipid and 50% protein (primarily apoproteins A-I and A-II) and are synthesised in the liver and intestine in nascent (precursor) form but fully develop in the plasma (aided by interaction with VLDL and chylomicrons). High density lipoproteins can be further divided into various subfractions, the principal ones being HDL₂ and HDL₃. The HDL₂ subfraction is larger and less dense than HDL₃ and although the major portion of HDL is normally present in HDL₃, individual variability in HDL levels in humans usually reflects different amounts of HDL₂ (Tall, 1990). The HDL particles circulate with a half-time of 5 to 6 days which is the longest of all the lipoproteins (Brown et al. 1981).

2.3.3 Apolipoproteins (apoproteins)

Aside from their role of providing structural stability to the lipoprotein molecules apoproteins also act as recognition sites for cell membrane receptors and function as cofactors for enzymes which are involved in plasma lipid and lipoprotein metabolism (Gotto, 1989). According to Brewer and colleagues (1988) fourteen major apoproteins have now been identified and characterised, these are classed A, B, C, D, E, F, G and H with subclasses. Of these fourteen, ten are commonly discussed in articles on lipids and lipoprotein metabolism and these are apoprotein (Apo) A-I, A-II, and A-IV; Apo B-48 and B-100; Apo C-I, C-II and C-III; Apo D and Apo E. The importance and function of these apoproteins will now be highlighted.

Apoprotein A-I is synthesised in both the liver and the small intestine and is the major protein present in HDL, constituting 70-80% of the protein mass. It is also present in chylomicrons. One of the major roles of Apo A-I is as an activator of the enzyme lecithin: cholesterol acyltransferase (see below) (Rosseneu and Labeur, 1990). Apoprotein A-I may also interact with a proposed HDL receptor and facilitate the removal of cholesterol from peripheral cells for transport back to the liver (Brewer et al. 1988). Apoprotein A-II is the second most abundant apoprotein in HDL and is also synthesised in both the liver and small intestine. Although its physiological role has not been
well established it has considerable lipid binding ability (Roheim, 1986). Apoprotein A-IV is synthesised in the intestine and the majority of Apo A-IV in the plasma is unassociated with lipoproteins. No definite function has yet been attributed to this apoprotein but it may aid in the activation of lecithin: cholesterol acyltransferase (Ginsberg, 1990).

There are two B apoproteins, Apo B-48 and Apo B-100. Apoprotein B-48, which is 48% as large as Apo B-100, is the major structural component of chylomicrons and is formed in the intestine. Apoprotein B-100, synthesised in the liver, is the largest of the apoproteins containing 4,563 amino acids and is present in VLDL, IDL and LDL (Brown and Goldstein, 1987). Apoprotein B-100 is an extremely important apoprotein since it is virtually the sole protein component of LDL and it is the ligand that delivers cholesterol to all parts of the body by the LDL receptor pathway (see below) (Assmann, 1982).

The C apoproteins are present in varying amounts in practically every lipoprotein class and are all synthesised in the liver. At present only Apo C-III has a clearly established physiologic function which is as an activator of the enzyme lipoprotein lipase (Mahley, 1991). Apoprotein D (sometimes termed Apo A-III) is a minor component of HDL and its site of synthesis is thought to be the liver. Its role is yet to be confirmed but may involve the transfer of cholesterol ester from HDL to triglyceride rich lipoproteins (Ginsberg, 1990).

Finally, Apo E, which is present in all of the lipoproteins, is synthesised primarily in the liver but also by various other cells including the brain (Gotto, 1989). Together with Apo B-100, Apo E acts as a ligand for the LDL receptor (sometimes referred to as the Apo B,E or Apo B,E [LDL] receptor). This means that it is responsible for the recognition of lipoproteins by the specific cell-surface receptors that mediate the uptake of lipoprotein cholesterol into the cells. It may also interact with the putative chylomicron remnant receptor (Apo E receptor) which is thought to play an important role in the removal of chylomicron remnants by the liver (Mahley, 1991; Brewer et al. 1988).

2.3.4 Enzymes involved in lipoprotein metabolism

Three key enzymes which are involved in lipoprotein metabolism are lipoprotein lipase, hepatic lipase and lecithin: cholesterol acyltransferase (LCAT). Lipoprotein lipase is bound to the endothelial surface in adipose
tissue and muscle and serves to hydrolyse circulating triglycerides to free fatty acids and glycerol. The concentration of lipoprotein lipase in the general circulation is kept low by an avid uptake in the liver thus lipoprotein lipase facilitates the movement of fatty acids to peripheral cells. Patients deficient in lipoprotein lipase or in its activator apoprotein (Apo C-II) demonstrate a massive hypertriglyceridaemia (Olivecrona and Bengtsson-Olivecrona, 1990). Hepatic lipase is responsible for the hydrolysis of triglycerides and phospholipids in HDL particles and is therefore involved in the catabolism of HDL. Thus, there is a strong negative correlation between hepatic lipase activity and levels of HDL, particularly HDL₂ (Olivecrona and Bengtsson-Olivecrona, 1990). Lecithin: cholesterol acyltransferase (activated by Apo A-I) is the enzyme which promotes the esterification of cholesterol on the surface of HDL. The cholesterol esters formed in this reaction are then incorporated into the hydrophobic core of HDL. Lecithin: cholesterol acyltransferase, then, plays a crucial role in HDL metabolism since it is responsible for converting the nascent HDL formed in the liver and intestine into its complete form in the plasma. Any reduction in LCAT activity and/or increase in hepatic lipase activity could theoretically reduce plasma HDL concentrations (Eisenberg, 1984).

2.3.5 Lipid digestion

Lipids undergo no significant digestion in the stomach and thus their digestion begins in the small intestine where they encounter the action of bile salts secreted from the liver. Bile salts are amphipathic molecules having a polar and nonpolar surface. The nonpolar surface of the bile salts associates with the nonpolar surface of the lipids, leaving the polar side exposed to water. Mechanical agitation in the intestine breaks up large fat globules and the resulting smaller droplets become coated with bile salts. Aggregation of the droplets is prevented since the negative charges on the exposed surfaces of the bile salts repel each other. The resulting suspension of lipid droplets in an aqueous solution is termed emulsion.

In this emulsified form, fat globules are then hydrolysed into monoglycerides and free fatty acids by the action of pancreatic lipase. The products of this hydrolysis now aggregate into micelles, which are similar in structure to emulsion droplets but much smaller and consist of bile salts, fatty acids, monoglycerides and phospholipids. These micelles facilitate the absorption of
lipids from the intestinal lumen into the epithelial cells lining the small intestine as described by Vander, Sherman and Luciano (p.523, 1990).

During their passage through epithelial cells fatty acids and monoglycerides are resynthesised into triglycerides, a process occurring in the endoplasmic reticulum where the enzymes for triglyceride synthesis are located. These new triglycerides, along with absorbed cholesterol and phospholipids, are encapsulated into globules with a protein coat forming chylomicron particles. The chylomicrons are now expelled from the epithelial cells and pass into the lacteals from where they reach the lymph. The lymph from the small intestine eventually empties into the left subclavian vein in the neck through the thoracic duct and thus gains access to the systemic circulation (Vander, Sherman and Luciano, 1990; Brooks and Fahey, 1984; Tortora and Anagnostakos, 1981).

2.3.6 Lipoprotein transport

Before they can be used by cells, triglycerides and cholesterol esters (esters of cholesterol and long chain fatty acids) must be hydrolysed to liberate fatty acids and unesterified cholesterol respectively. By means of the lipoprotein transport system these two classes of hydrophobic lipids are delivered to their separate destinations; triglycerides to adipose tissue and muscle where the fatty acids can be stored or oxidised for energy and cholesterol esters to all body cells where the unesterified sterol can be used in the construction of plasma membranes. It should be pointed out that almost all cells can synthesise some of the cholesterol required for their own plasma membranes, but most cannot do so in sufficient quantities and thus depend on receiving cholesterol from plasma LDL (Vander, Sherman and Luciano, 1990). The lipoprotein transport system can be divided conceptually into exogenous and endogenous sections involved in the transport of dietary and hepatic lipids respectively. What follows is a brief summary of these two systems which are described in more detail elsewhere (Assmann, 1982; Betteridge, 1989; Brewer et al. 1988; Brown and Goldstein, 1984; Brown et al. 1981; Eisenberg, 1990; Ginsberg, 1990; Tikkanen, 1990).

Exogenous lipid transport: Dietary lipids are digested and packaged into triglyceride rich chylomicrons as described above. The major function of these chylomicrons is to transport the dietary lipids from the intestine to peripheral
tissues and the liver. The principle protein components of chylomicrons in the lymph are Apo A-I, A-II, A-IV and Apo B-48. Once chylomicrons have gained access to the bloodstream they acquire other apoproteins from HDL including Apo C-II and E. Apoprotein C-II activates the lipoprotein lipase enzyme residing in the capillaries of adipose and muscle tissues. This enzyme partially hydrolyses the triglycerides contained in the chylomicron to monoglycerides and free fatty acids and allows them to gain access to muscle or fat tissues. The chylomicron also gives up the excess phospholipids, free cholesterol and apoproteins contained on its surface, these are transferred to HDL. As much as 50% of the circulating HDL particles may be derived from this process (Betteridge, 1989). The chylomicron retains its load of cholesterol ester and Apo E.

Depleted of its triglyceride core, the chylomicron remnant (as it now becomes known) is released from the capillary wall and re-enters the circulation to be carried to the liver where it is internalised, off loading its cholesterol. The internalisation of the chylomicron remnant by the liver is accomplished via a process termed 'receptor mediated endocytosis' (Brown et al. 1981) and is thought to involve a remnant receptor with an affinity for Apo E and perhaps the LDL receptor as well. The liver disposes of the delivered cholesterol in bile, either as unesterified cholesterol or as bile acids. A large portion of this cholesterol and bile acid is secreted by the liver, reabsorbed in the intestine and then again delivered to the liver, thus forming a continuous cycle. Some cholesterol and bile acid avoids reabsorption, however, and is excreted in the faeces.

Endogenous lipid transport: Carbohydrates and fatty acids are converted into triglycerides in the liver. In addition the liver synthesises its own supply of cholesterol, via the activity of the rate controlling enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase), to complement its dietary sources. Triglycerides and cholesterol in the liver are packaged into VLDL for their transport to adipose tissue and extrahepatic cells respectively. Once in the circulation VLDL acquires Apo E and Apo C-II from HDL and then surrenders most of its triglyceride when it encounters lipoprotein lipase activity in the capillaries of adipose tissue. The interaction of VLDL with lipoprotein lipase is not as effective as the interaction of chylomicrons with this enzyme, thus, the half life of VLDL is one to three hours in contrast to that of four to five
minutes for chylomicrons (Brown et al. 1981). Some of the VLDLs are removed from the circulation by way of Apo B-100/Apo E interaction with LDL receptors located on the liver. The remaining particles are converted into IDL. During this process excess surface material (proteins, phospholipids and cholesterol) are passed to HDL which esterifies the excess cholesterol with fatty acids, via interaction with the enzyme LCAT and transfers it back to IDL. The net result of this conversion is the replacement of most of the triglyceride core of VLDL with cholesterol esters.

After lipolysis, IDLs are released from the capillary wall back into the circulation and some are returned to the liver where they are catabolised. Most, however, undergo a further transformation in which they are converted into LDL. The conversion process is not yet fully understood but involves the removal of all of the remaining triglyceride and most of the apoproteins from IDL leaving a particle which contains almost pure cholesterol ester in its core and Apo B-100 at the surface. The LDL, which has an average lifespan of two and a half days (Brown and Goldstein, 1984) delivers cholesterol to extrahepatic cells and the liver. Delivery is accomplished when the LDL binds to high affinity receptors located on the surface of hepatic and peripheral cells and is internalised by endocytosis as described by Brown and Goldstein (1984). If the circulating plasma levels of LDL are high other methods for the removal of this lipoprotein can be utilised including the degradation of LDL by 'scavenger cells'. However, if these scavenger cells are overloaded with cholesterol they become converted into 'foam cells' which form components of atherosclerotic plaques as described below.

The extrahepatic and scavenger cells themselves excrete a significant portion of cholesterol as a result of cell death and membrane turnover. This cholesterol is collected by HDL, again in conjunction with the action of LCAT, and transferred back to the liver, either directly, or via exchange with VLDL, IDL and LDL. This transfer of cholesterol esters from HDL back to other lipoproteins is thought to be aided by the action of an enzyme termed cholesterol ester transfer protein (Barter, 1990a). The involvement of HDL in returning cholesterol from extrahepatic cells to the liver may involve specific HDL receptors located on peripheral cells and perhaps also on the liver. Moreover, unlike LDL, HDL is not thought to be catabolised by these receptors but is released back into the circulation after delivery/collection of cholesterol esters so that further transport of cholesterol may continue. However, at
present this process is not well understood (Tall, 1990). It is clear though that HDL plays a central and quite unique role in the continuous cycle of lipoprotein transport.

2.3.7 Low density lipoprotein receptors, the liver and regulation of plasma cholesterol

The liver can be considered as the "centre of the cholesterol universe" since it may be simultaneously synthesising new cholesterol, picking cholesterol up from the blood and excreting it into bile and converting cholesterol into bile acids (Vander, Sherman and Luciano, 1990). Regulation of plasma cholesterol concentration is attained by manipulation of all of these processes the most crucial one being control of cholesterol production. The rate of hepatic cholesterol synthesis is highly responsive to the quantity of absorbed exogenous cholesterol. Thus, an increase in the intake of dietary cholesterol will lead to a reduced hepatic output and vice versa. This control mechanism is achieved by changes in the activity of HMG CoA reductase (Stryer, 1988).

A key role in the control of plasma cholesterol levels is performed by the LDL receptors. Uptake of cholesterol by these receptors results in a cellular increase in free cholesterol which activates three regulatory mechanisms to oppose further increases; 1: The synthesis of HMG CoA reductase is suppressed; 2: The activity of acyl coenzyme A: cholesterol acyltransferase is enhanced so that excess cholesterol can be stored in the form of cholesterol esters, and; 3: The synthesis of LDL receptors is inhibited, thus preventing a further uptake of cholesterol by the cells and therefore protecting them from an over accumulation of cholesterol (Assmann, 1982. Stryer, 1988).

The fundamental importance of the role of LDL receptors in the regulation of plasma cholesterol levels is demonstrated in those people who have a genetically inherited defect in LDL metabolism. This defect usually manifests itself in a marked reduction in the number of LDL receptors located on hepatic and peripheral cells, entry of LDL into the cells is impaired as a consequence and the plasma level of LDL rises. This condition is termed familial hypercholesterolaemia and there are two forms of the disease, a heterozygous form and a more severe homozygous form. Heterozygotes (approximately one in 500 people) have one nonfunctional gene and one normal gene in each cell for LDL receptor synthesis, thus they produce about
half the normal number of receptors and exhibit plasma LDL levels twice that in normal people even before birth. Homozygotes (about one in a million people) inherit two abnormal genes, one from each parent, and produce virtually no functional LDL receptors at all. They usually have circulating LDL levels which are six times greater than normal and most homozygotes die of CHD in childhood (Ball and Mann, 1988; Stryer, 1988; Brown and Goldstein, 1984).

2.3.8 Cholesterol, atherosclerosis and coronary heart disease

The underlying cause of CHD in most cases is the formation, over many years, of atherosclerotic plaques. These plaques are rich in cholesterol, most of which is derived from plasma lipoproteins (Grundy, 1990a). The precise mechanism responsible for the development of these plaques has yet to be resolved but many theories have been proposed (see Davies et al. 1991; Getz, 1990; Havel, 1989; McGill, 1988; Woolf, 1990). Briefly, cholesterol and blood platelets penetrate the walls of one or a number of arteries, possibly prompted by endothelial injury, and are degraded by smooth muscle cells and macrophages (white blood corpuscles sited in the walls of blood vessels) to form what are termed foam cells (so named because of their microscopic appearance). These cells contain a critical concentration of cholesterol esters which ultimately leads to their death. If the circulating level of plasma cholesterol is too high, cholesterol accumulates in and among the foam cells causing an 'atheroma' or atherosclerotic plaque. This narrows the artery and may eventually rupture releasing thrombotic material which causes total occlusion of the artery and thus heart attack.

Since all of the plasma lipoproteins exhibit different structural and functional roles each lipoprotein class is thought to be differently related to atherosclerosis. What follows is a brief examination of the role of each of the major lipoprotein species in the development of atherosclerosis.

2.3.9 Low density lipoproteins and atherosclerosis

Low density lipoproteins are generally thought to be the major atherosclerotic lipoproteins. The Lipid Research Clinics Coronary Prevention Trial (Lipid Research Clinics Programme, 1984) and many other epidemiological studies (see Grundy, 1990a) have revealed a strong and independent relationship
between plasma concentrations of LDL and the risk of developing atherosclerotic heart disease. Perhaps the most compelling evidence implicating LDL in the development of CHD, however, comes from the genetically determined condition homozygous familial hypercholesterolaemia. This "vivid experiment of nature... demonstrates unequivocally the casual relation between an elevated circulating LDL level and atherosclerosis" (Brown and Goldstein, 1984). Furthermore, direct evidence linking LDL with CHD has now been provided from studies in which LDL like lipoproteins have been extracted from atherosclerotic plaques (Getz, 1990).

Recent studies suggest that certain subclasses of LDL may be more atherogenic than others. One of these (LDL-III) is characterised by relatively small particle size and increased density and has been found to be linked to many interrelated hormonal and metabolic factors also implicated in CHD risk (Krauss, 1991). Furthermore, the atherogenicity of LDL appears to be increased by oxidisation (Luc and Fruchart, 1991; Olsson, 1990). Oxidised LDL gains access to macrophages via the scavenger receptor pathway, which, unlike the LDL receptor is not subject to feedback regulation (McGill, 1988; Betteridge, 1989). Thus, the higher the concentration of circulating LDL, the higher its influx into the arterial wall (Stender, 1990).

2.3.10 High density lipoproteins and atherosclerosis

Interest in the role of HDL in the aetiology of CHD was heightened when Miller and Miller (1975) published a "rediscovery" of the inverse relationship between this lipoprotein and heart disease first proposed by Barr and co-workers in 1951 (cited in Jacobs et al. 1990). A subsequent study found HDL cholesterol to be a three-fold better predictor of CHD than LDL cholesterol in a relationship which was independent of all other variables (including LDL cholesterol) (Miller et al. 1977). These findings have subsequently been confirmed in a number of large clinical and population based North American and European studies (Frick et al. 1990; Gordon et al. 1989; Jacobs et al. 1990). Moreover, although the British Regional Heart Study found that HDL cholesterol was not a major risk factor for ischaemic heart disease in British men (Pocock et al. 1986), this inconsistency arose from a "statistical artefact" which has since been corrected yielding results consistent with other studies (Gordon and Rifkind, 1989).
In addition to epidemiological studies, evidence supporting an inverse relationship between HDL and CHD is provided by investigations examining patients with genetic disorders such as familial hyperalphalipoproteinaemia. This disease was first reported by Glueck and co-workers (1975) and later Patsch and colleagues (1981) and is characterised by elevated levels of serum HDL cholesterol and low morbidity and mortality from CHD. Angiographic studies have also demonstrated that HDL concentrations are negatively associated with the development of CHD (Miller et al. 1990) and positively related to increased longevity (Nikkila and Heikkinen, 1990).

Two main theories have been proposed to explain the seemingly protective role of HDL in preventing CHD. The most popular of these proposes that HDL collects cholesterol from LDL, peripheral tissues and the arterial wall and returns it to the liver for disposal in a process termed 'reverse cholesterol transport' (see Barter, 1990b; Kottke, 1986; Miller 1990; Roheim, 1986). High density lipoprotein is a good acceptor of unesterified cholesterol from cell membranes and a high concentration of HDL within the walls of coronary arteries could mobilise cholesterol out of foam cells (Grundy, 1990a). This is supported by evidence that the cholesterol content of healthy vascular tissue is a negative function of plasma HDL cholesterol in humans (Miller, 1990). It has also been shown recently that there is a net transfer of cholesterol esters from LDL to HDL in the plasma of normolipidaemic humans (Van Tol et al. 1991). The level of HDL cholesterol in the plasma then is seen as a marker for the efficiency of the system, with low levels indicating a reduced capacity for cholesterol removal and thus increased peripheral cholesterol deposition. However, although the reverse cholesterol transport hypothesis is attractive, evidence to support it is limited and many precise details related to its proposed workings remain to be elucidated (Barter, 1990b).

Another theory proposed by such investigators as Lechleitner and associates (1990) and Gotto (1990) does not involve HDL in such a direct role. These authors emphasise the strong inverse relationship between HDL and triglycerides and argue that the two cannot be considered separately. Thus, it is suggested that a rapid clearance of triglycerides (perhaps due to increased lipoprotein lipase activity) promotes the formation of HDL2 and that the low levels of triglyceride rich lipoproteins reduce the transfer of HDL cholesterol esters into these lipoproteins. Therefore, HDL cholesterol is kept high reducing the amount of cholesterol available for incorporation into lipoprotein
subfractions associated with increased CHD risk. Conversely, delayed clearance of triglyceride rich lipoproteins prevents the formation of HDL2 and leads to enhanced loss of cholesterol esters into atherogenic lipoproteins (Lechleitner et al. 1990). Once again, however, more experimental evidence is required to substantiate this theory.

In conclusion then, despite overwhelming evidence linking increased levels of HDL cholesterol to reduced CHD risk, the mechanisms of interaction are still uncertain. Moreover, it remains to be shown definitively whether high HDL levels are predominantly causative or coincidental in their relationship to reduced CHD risk.

2.3.11 Triglycerides and atherosclerosis

Research findings examining the relationship between triglycerides and CHD are still largely ambiguous as shown in a recent review by Austin (1991). This ambiguity may be related to many factors. Triglyceride levels correlate positively with LDL cholesterol and inversely with HDL cholesterol and these correlations could confound the triglyceride-CHD link (Grundy, 1990b). Although epidemiological studies have often found an association between triglyceride concentration and the incidence of CHD when univariate statistical analyses are employed, this relationship frequently loses significance when multivariate analyses are employed (Gordon, 1990). Thus, the role of plasma triglycerides as an independent risk factor for CHD remains uncertain but has not been discounted since CHD risk is increased in some genetic disorders resulting in hypertriglyceridaemia without simultaneous hypercholesterolaemia (Havel, 1990).

The complexity of this issue is increased by the fact that a number of triglyceride rich lipoproteins are responsible for the transport of triglyceride within the body. These include chylomicrons, chylomicron remnants, VLDLs and VLDL remnants. Evidence certainly exists to suggest that at least some of these lipoproteins are atherogenic (see Grundy, 1990a; Schwandt, 1990 and Ginsberg, 1990) but their quantitative importance in cases where other risk factors are also elevated is unknown. Much further study is needed to resolve this issue and will require measurement of the postprandial concentrations of the various triglyceride rich lipoproteins rather than their post absorptive concentrations. Indeed it is possible that much important information
concerning coronary risk has been missed due to the common practice of collecting plasma only in the postabsorptive state (Pearson, 1991).

2.3.12 Lipoprotein(a) (Lp(a))

Current understanding of Lp(a) structure and metabolism is described in several recent reviews (Lawn, 1992; Loscalzo, 1990; Scanu, 1990; Utermann, 1990). Lipoprotein(a) is an LDL-like particle which is heterogeneous in size and density and contains a single copy of Apo B-100 linked to a high molecular mass glycoprotein called apoprotein(a). This Apo B-100/apoprotein(a) combination occurs in the plasma either in lipid free form, bound to cholesterol rich lipoproteins, or in association with triglyceride rich like particles. The major site of apoprotein(a) synthesis is the liver but it is not yet known whether apoprotein(a) combines with Apo B-100 in the liver or the plasma to form Lp(a). The site and mechanism of Lp(a) catabolism has still to be determined though the LDL receptor may be involved since Lp(a) contains Apo B-100. The concentration of Lp(a) in the plasma is thought to be determined largely by genetic factors (Boerwinkle, 1992). The physiological function of Lp(a) remains uncertain although it is probably related to lipid transport, blood clotting or both.

Since its discovery in 1963 by Kare Berg many studies have demonstrated that high levels of Lp(a) are related to CHD (Boerwinkle, 1992; Dahlen et al. 1986; Genest et al. 1991). Moreover, there is currently much interest in Lp(a) since many feel that it will provide a link between atherosclerosis and thrombosis. This hope comes from the fact that there is a striking similarity between apoprotein(a) and plasminogen regarding particular configurations called kringles which enable molecules to bind to cells. Plasminogen is a precursor of plasmin which is capable of decomposing fibrin and therefore dissolving blood clots. Because of its similarity with plasminogen, Lp(a) is thought to act as an invader in the fibrinolytic system, interfering with fibrinolysis in many ways as described by Miles and Plow (1990) and thus promoting thrombotic events.

Although the evidence is still controversial it has been proposed that Lp(a) penetrates the arterial wall following endothelial damage and binds to fibrin. This may be beneficial since Lp(a) would provide cholesterol to the site of tissue injury. If the concentration of Lp(a) was too high, however,
'microthrombi' may be formed on the arterial wall and become incorporated into atherosclerotic plaques (Brown and Goldstein, 1987). Moreover, Lp(a) may inhibit the action of plasminogen by preferentially binding to the plasminogen receptors located on endothelial cell surfaces, thus hindering the cleavage of fibrin clots (Lawn, 1992). It has also been suggested that Lp(a) might be involved in the final acute event in atherosclerosis where an atheroma becomes 'active' and ruptures (Loscalzo, 1990). At present, however, none of the above hypotheses have been proved and it cannot be excluded that Lp(a)'s homology with plasminogen is merely coincidental and unrelated to its atherogenic role. If this is the case the atherogenicity of Lp(a) may be related to the covalent linkage formed between apoprotein(a) and Apo B-100 which could prevent uptake of Apo B-100 containing particles via LDL receptors. As a consequence Lp(a) would be diverted to the scavenger receptor pathway leading to intracellular accumulation and the transformation of macrophages into foam cells (Scanu, 1990; Breckenridge, 1990).

To conclude then, Lp(a) may be involved in both the clinically quiescent evolutionary development of atherosclerosis and the clinically active acute phase of atherosclerosis via interference in the fibrinolytic system. It could be, however, that Lp(a) is atherogenic only on the basis of its properties as a lipoprotein or that Lp(a) disrupts both lipoprotein metabolism and fibrinolytic activity.

2.3.13 Apoproteins A and B and coronary heart disease

Recent trends in the assessment of CHD risk include measurements of the apoproteins A-I and B due to their relationships with HDL and LDL cholesterol respectively. In some studies the discriminative value of Apo A-I in detecting CHD has been shown to be superior to that of HDL and other lipoproteins (Breckenridge, 1990; Durrington, 1989). Similarly Apo B may be more strongly related to the risk of atherosclerosis than LDL cholesterol (Durrington, 1989; Krauss, 1991). At present it is not known whether the predictive powers of apoproteins A-I and B in detecting atherosclerosis are related simply to their role as markers for HDL and LDL cholesterol or whether they are directly atherogenic themselves.
2.4 Variabilities in lipid and lipoprotein measurement

Interest into the problems inherent in lipid and lipoprotein measurement has proliferated recently due to the publication of guidelines for the diagnosis and treatment of hypercholesterolaemia in Britain (Shepherd et al. 1987), Europe (Study Group: European Atherosclerosis Society, 1987) and North America (Report of the National Cholesterol Education Programme, 1988). Clearly inaccuracies in the measurement of serum lipids and lipoproteins have serious implications for cholesterol screening programmes and also individual patients, but factors which may confound lipid and lipoprotein determinations are also of great interest and relevance to the research scientist who relies on accurate and reproducible data. The factors which may cause the concentration of a given lipid or lipoprotein to vary between different samples collected from the same individual can be divided into three main areas: biological variation, analytical variation and variation due to sampling and handling procedures.

Variation due to sampling and handling may be caused by many factors which have been reviewed by Cooper and colleagues (1988). In particular changes in posture and the length of venous occlusion may cause inconsistent results. Standing increases serum cholesterol concentration while levels are decreased in the recumbent position and changes of up to 10 to 15% may occur depending on the period of recumbency (Breckenridge, 1990). These differences are due to a rapid and progressive haemodilution and haemoconcentration caused by moving to and from supine and standing positions respectively. Prolonged application of a tourniquet also results in a haemoconcentration since it increases the pressure within the veins and causes serum water to move into interstitial spaces. Venous occlusions for less than one minute are unlikely to affect the concentration of serum lipids but longer periods of occlusion may result in serum lipid elevations of up to 15%. Other factors which may cause pre-analytical variation are the type of collection tube used, the addition of anticoagulants to samples and differences in storage and shipping procedures (Cooper et al. 1988).

There are two important components related to analytical variation: accuracy and precision. Accuracy, agreement with the true value, is a measure of any inherent bias in an analytical procedure when compared to a gold standard for the determination of a particular lipid or lipoprotein. Precision can be defined
as the ability of an analytical procedure to reproduce a result regardless of whether or not that result represents a true value (Breckenridge, 1990). Both within day and day-to-day precision are extremely important since constant accuracy is not possible if the measurements are imprecise (Naito, 1988).

Analytical imprecision may be expressed as the coefficient of variation (CV) and this can be used to calculate the possible range of values which may be obtained when analysing a serum sample for a particular lipid or lipoprotein. If a cholesterol assay has a CV of 10%, for example, repeated measurements of a sample with a 'true value' of 6.2 mmol.l⁻¹ would result in a spread of values 95% of which would fall within the range of 5.0 - 7.4 mmol.l⁻¹ (Warwick et al. 1990). Clearly such an imprecise method of cholesterol analysis would be undesirable. Naito (1988) states that for total cholesterol a CV of 5% is acceptable while a CV below 3% would be ideal. Yet even with these levels of precision the 95% range would span from 5.6 to 6.8 mmol.l⁻¹ and from 5.8 to 6.6 mmol.l⁻¹ respectively given a 'true value' of 6.2 mmol.l⁻¹ (See Figure 2.2).

Figure 2.2 The effect of analytical imprecision on the range of cholesterol values reported at a 'true value' of 6.2 mmol.l⁻¹ (from Warwick et al. 1990).
Measurements of other lipids and lipoproteins are confounded by further sources of analytical error. For example, the potential for analytical variation is increased during HDL analysis because of the precipitation procedure and triglyceride measurements which are not corrected for free glycerol will obviously result in a positive measurement bias (Naito, 1988). Finally, analytical variation is usually greater for apolipoproteins than it is for lipoproteins due to the inherent problems with immunoassays (see Cooper et al. 1988). Although duplicate analysis will reduce the amount of analytical variation to some extent this should not be seen as an alternative to improving the accuracy and precision of measurement procedures and a total analytical variability of below 3% should be aimed for (Von Schenck and Olsson, 1990).

Despite the potential sources of error discussed above, in most cases biological variation has been shown to account for the majority of the total variability which occurs in lipid and lipoprotein assessment. Natelson and co-workers (1988) measured serum cholesterol concentrations in one subject over a six hour period by sampling blood every 20 minutes. The assay CV was below 1.5% but cholesterol concentration varied from a low of 4.9 mmol.l⁻¹ to a peak of 5.5 mmol.l⁻¹. Moreover, Bremner and colleagues (1990) have studied the biological variation of total cholesterol, HDL-C and triglycerides over a 24 hour period in nine subjects who gave blood every three hours. Mean values for the group ranged from 4.6 to 5.9 mmol.l⁻¹ for total cholesterol, from 0.9 to 1.2 mmol.l⁻¹ for HDL-C and from 1.6 to 3.4 mmol.l⁻¹ for triglyceride. More profound differences were found when examining individual data and in one subject total cholesterol concentration varied from 3.5 to 5.3 mmol.l⁻¹ over the course of the 24 hours.

Other investigators have examined the variation in lipids and lipoproteins over longer periods. Bookstein and associates (1990), for example, measured serum lipids in 51 men and women three times in one week on an every other day schedule. They found a day-to-day variability of 5% for total cholesterol, 10% for HDL-C, 20% for triglyceride and 8% for calculated LDL-C. In each case the biological variation was greater than the analytical variation although analytical variation did contribute significantly to the total variation in HDL-C and LDL-C. Mogadam and colleagues (1990) assessed weekly variation in the serum lipid levels of 20 male and female subjects by collecting samples once a week for four consecutive weeks. Variations of more than ±20% in the concentrations of total cholesterol, LDL-C (calculated) and HDL-C were seen
in 75%, 95% and 65% of the subjects respectively and triglyceride concentrations varied by more than ±30% in 95% of the subjects. In all cases biological variation was by far the most significant component of the total variation. Finally, Demacker and co-workers (1982) examined the variation in serum lipids and lipoproteins over a period of one year in 28 men. Biological variation (expressed as the CV of all measurements within a person) ranged from 3.9% to 10.9% for total cholesterol, from 5.6% to 12.4% for HDL-C and from 12.9% to 39.9% for triglycerides.

Few studies have investigated the intra-individual variation of apolipoproteins or HDL-C subfractions but some data is available. Wasenius and co-workers (1990) reported a daily biological variation of 6.5% for both Apo A and Apo B while the monthly values increased to 9.4% and 9.7% respectively. Brown and colleagues (1990) have reported slightly higher values of 10.4% and 12.2% for the biological variation of Apo A-I and Apo B over a six month period. In this same study Brown and colleagues (1990) also measured HDL₂-C and HDL₃-C and found an intra-individual variation of 18.6% and 16.0% respectively.

The causes of the biological variation in serum lipids and lipoproteins in the short term are not well understood but they may be related to spontaneous fluctuations in metabolism (Rotterdam et al. 1987). It has also been suggested that circadian rhythms may explain the daily variation in lipids and lipoproteins. However, experimental evidence does not support this suggestion except in the case of triglycerides which have been shown to vary significantly according to the time of day but this is usually related to the proximity of meals (Bremner et al. 1990; Demacker et al. 1982; Wasenius et al. 1990). In the longer term a whole host of factors may explain fluctuations in lipids and lipoproteins including body weight changes and changes in dietary, smoking, alcohol and exercise habits, the development of underlying disease and seasonal variation (Breckenridge, 1990; Cooper et al. 1988; Durrington, 1990).

It is clear from the above discussion that biological and analytical variation can have a substantial influence on lipid and lipoprotein determinations. Just how profound this influence can be has been demonstrated by Hyltoft Petersen and colleagues (1990). In a project on risk factors for CHD they measured serum cholesterol in three specimens from each subject drawn at two to three
week intervals. In 100 of their patients the measured cholesterol concentrations were between 7.0 and 8.0 mmol.l\(^{-1}\) in the first sample. However, repeat analysis resulted in a spread of values which ranged from 5.0 to 10.0 mmol.l\(^{-1}\) by the third sample despite an assay CV of less than 1% at a concentration of 7.5 mmol.l\(^{-1}\) (see Figure 2.3). If this had been an intervention trial a decrease in cholesterol concentration in any given individual could easily have been wrongly attributed to therapeutic success. Although this problem cannot be completely overcome it is vital that sampling procedures are rigourously standardised and that assay inaccuracy and imprecision are reduced to a minimum. Furthermore, several specimens need to be collected to reduce biological variation and it may take as many as six samples to estimate an individual's cholesterol value to within 5% of the true mean value (Rotterdam et al. 1987).

![Bar chart of cholesterol values](image)

**Figure 2.3** Cholesterol values in the third of three samples taken from 100 individuals, where the first sample had values between 7.0 and 8.0 mmol.l\(^{-1}\) (adapted from Hyltoft Petersen et al. 1990).
2.5 Exercise, lipids and lipoproteins

2.5.1 Introduction

Interest in the effects of exercise on lipoproteins has proliferated in recent years due to the finding that exercise may exert favourable changes on plasma lipoprotein concentrations. Although there is little direct evidence to suggest that exercise can independently alter plasma total cholesterol or LDL-C, there is strong evidence to support the hypothesis that exercise, more especially endurance type exercise, is an effective means of increasing HDL-C and thus reducing the risk of CHD.

Some of the research findings related to exercise and lipoproteins must be interpreted with a certain amount of caution, however, due to factors related to poor study design. An inadequate number of participants and the omission of a control group are fundamental flaws in some training studies. Moreover, cross-sectional studies comparing the lipoprotein profiles of groups of active and inactive people have often failed to control for the effects of such variables as diet, smoking habits, alcohol consumption and body composition, all of which are known to have an effect on lipoproteins (Goldberg and Elliot, 1987). Inappropriate use of statistical procedures has also been "extensive" which makes the conclusions of many studies unfounded (Wood et al. 1984).

Other confounding factors related to findings on exercise and lipoprotein metabolism are the differing mode and duration of exercise examined. Thus, longitudinal training studies have been conducted for as little as six weeks (Lipson et al. 1980) to as long as two years (Wood et al. 1985) and exercise studies of various kinds have investigated such diverse activities as brisk walking (Hardman et al. 1989a), endurance running (Williams et al. 1982), cycling (Giada et al. 1988), aerobic dance training (Williford et al. 1988), volleyball playing (Bonetti et al. 1988), endurance triathlon (Lamon-Fava et al. 1989), speed skating (Farrell et al. 1982), tennis playing (Vodak et al. 1980) and weight training (Goldberg et al. 1984). Variations in the frequency and intensity of the exercise performed in these studies is another factor adding to the complexity of the issue and when taken together all of the above observations make generalisation of research findings into exercise and lipoprotein metabolism difficult and sometimes confusing. Nevertheless, some
conclusions can be drawn from the available research and these will be discussed here.

2.5.2 Exercise, total cholesterol and low density lipoprotein cholesterol

Haskell (1986) and Goldberg and Elliot (1987) have conducted fairly extensive reviews of the literature concerning exercise and lipids and both conclude that the evidence for an independent effect of exercise on total cholesterol and LDL-C is limited. Although some observational studies have shown lower levels of these variables in trained subjects, many have found no differences. Moreover, the results of longitudinal studies are no more consistent and when changes are found they are usually quite small (five to seven per cent) unless the exercise is accompanied by a substantial weight loss or change in dietary intake (Haskell, 1986).

On balance, the findings of more recent investigations are probably in line with the above conclusions but some are worthy of a mention. Studies by Hill and colleagues (1989) and Hespel and colleagues (1988a) examining asymptomatic males, Despres and co-workers (1990) with moderately overweight young men and Mendoza and associates (1991) investigating men with premature myocardial infarction, have all found reductions of around 11 to 15% in the concentration of total cholesterol and/or LDL-C after endurance training programmes lasting from between ten weeks to four months. Unfortunately, however, none of these studies used control groups and thus these changes are questionable.

Of the latest longitudinal studies that are well designed the majority have found no significant changes in total cholesterol or LDL-C after endurance exercise programmes. These include studies by Hardman and colleagues (1989a) examining a one year programme of brisk walking in middle-aged women; Marti and associates (1990) assessing the effects of four months of walking/jogging in middle-aged Swiss men and Sasaki and co-workers (1987) investigating the responses of obese children to a running schedule over a two year period. Recent cross-sectional studies, however, have continued to yield conflicting results with some finding no differences in total cholesterol and/or LDL-C between endurance trained athletes and their sedentary counterparts (Northcote et al. 1988; Tamai et al. 1988) and others.
finding lower values in the trained subjects (Giada et al. 1988; Higuchi et al. 1988).

In general then exercise does not dramatically alter total cholesterol or LDL-C. Nevertheless, some observational studies have found lower concentrations of these parameters in endurance trained subjects compared with their inactive counterparts (Giada et al. 1988; Higuchi et al. 1988). Furthermore, well designed and executed training studies have shown reductions in total cholesterol and LDL-C with endurance training (Brownell et al. 1982; Peltonen et al. 1981; Wood et al. 1985). It may be that the effects of exercise on total cholesterol and LDL-C are determined largely by pretraining levels. Merrill and Friedrichs (1990) examined the potential of endurance exercise to influence total cholesterol and LDL-C concentrations in groups of subjects with low (3.7 mmol.l⁻¹), medium (4.5 mmol.l⁻¹) and high (5.7 mmol.l⁻¹) concentrations of total cholesterol and found reductions in both variables after training but only in the group which had initially high values. Since studies rarely show increased concentrations of these variables in physically active people it might be concluded that endurance exercise has some small potential to improve lipoprotein profiles in this respect.

Some investigators are now examining the effects of exercise on LDL subfractions due to the finding that the smaller, denser LDL subfractions may be more atherogenic than other LDL subfractions (Krauss et al. 1987). It has been shown that distance runners have significantly lower concentrations of the smaller LDL subfractions than age matched non-runners (Williams et al. 1986) and that endurance training can lead to a reduction in the concentration of small LDL subfractions (Williams et al. 1990a). Moreover, in a one year training study, distance run correlated negatively with the concentration of small LDL although changes were not significantly different between the exercise and control group (Williams et al. 1989). However, additional research is required before any firm conclusions can be reached as to the potential of exercise to alter LDL subfractions.

2.5.3 Exercise and high density lipoprotein cholesterol

Results from studies examining the relationship between exercise and HDL-C have been more consistent than those examining total cholesterol or LDL-C and there is strong evidence supporting the hypothesis that endurance type
exercise is associated with increases of HDL-C. Wood and associates (1984) list the results of 14 observational running studies and eight observational studies examining activities other than running which have shown significantly higher HDL-C concentrations in active subjects as compared to inactive controls. More recently the results of many cross-sectional studies have confirmed these findings (Frey et al. 1990, Giada et al. 1988; Higuchi et al. 1988; Higuchi et al. 1989; Marti et al. 1991; Nagao et al. 1988; Northcote et al. 1988; Sady et al. 1988; Tamai et al. 1988). Although it is true that observational studies are not proof of an exercise effect, since they cannot discount the possibility that both HDL-C and exercise are associated with some secondary variable e.g. leanness or some other constitutional factor, they do provide evidence supporting an influence of physical activity on HDL-C concentration.

One particularly convincing observational study is that of LaPorte and co-workers (1983) who compared the HDL-C concentration of subjects with habitual exertion levels spanning a whole spectrum of physical activity from quadriplegia to marathon running. They found a graded relationship between activity and HDL-C with the lowest levels of both existing in newly injured spinal cord patients and progressive increases in both through groups of recently injured spinal cord patients, patients with long term spinal cord injuries, patients disabled with chronic back pain, normal sedentary controls, recreational joggers and finally elite marathon runners (Figure 2.4). Another well designed cross-sectional study is that of Nakamura and colleagues (1983). They compared a group of regular joggers with a control group matched for sex, age, bodyweight index, total cholesterol and triglycerides and found significantly higher (23%) concentrations of HDL-C among the joggers than the controls.
Although evidence from longitudinal studies is not as consistent as that from cross-sectional studies there are still many studies showing elevations of HDL-C with endurance training (Despres et al. 1990; Faria and Faria, 1991; Hardman and Hudson, 1991; Hespel et al. 1988b; Marti et al. 1990; Mendoza et al. 1991; Sasaki et al. 1987; Stein et al. 1990; Thompson et al. 1988; Wood et al. 1988). These have involved exercise training programmes of between 12 weeks (Stein et al. 1990) and two years (Sasaki et al. 1987) in length and have found increases in HDL-C of between 5.9% (Marti et al. 1990) and 27% (Hardman and Hudson, 1991). In contrast a number of the latest longitudinal studies have not shown increases in HDL-C with exercise (Hill et al. 1989; McNaughton and Davies, 1987; Palank and Hargreaves, 1990; Raz et al. 1988; Tanaka et al. 1988 and Williford et al. 1988).

Many factors could be responsible for the less consistent results obtained from longitudinal studies. Firstly, the quantity and quality of training achieved over a given period in these studies is usually considerably less than that of the trained subjects examined in cross-sectional studies. Moreover, many of the longitudinal studies are conducted over very short periods of time which possibly are not sufficient for changes in lipoproteins to occur and even in the

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Figure 2.4  High density lipoprotein cholesterol concentration in groups of subjects who have different habitual physical activity levels. SCI = Spinal cord injury. (Adapted from LaPorte et al. 1983).
longer studies the amounts of exercise performed do not approximate the quantities achieved by elite athletes and other sports participants in their many years of consistent training. Other reasons for the inconsistencies could be related to differences in the characteristics of the groups investigated. For example, Sutherland and Woodhouse (1980) have found that changes in HDL-C are often dependent on pretraining levels. Thus, subjects with initially low levels of HDL-C have a greater potential to increase their levels than subjects with high levels initially. Similarly, those with poor fitness at the outset of a study may have a greater potential to improve their fitness and therefore perhaps their HDL-C concentration, as has been shown (Hill et al. 1989).

In an effort to elucidate more precisely the relationship between exercise, HDL-C and CHD some investigations have measured the principle subfractions of HDL-C. In particular attention has focused on the HDL$_2$-C subfraction since this subfraction is thought to be largely responsible for the protective effects of HDL-C (Miller et al. 1981). This has proved fruitful and many studies, both observational and longitudinal, have shown increased concentrations of HDL$_2$-C with physical activity (Frey et al. 1990; Giada et al. 1988; Hespel et al. 1988b; Northcote et al. 1988; Stubbe et al. 1983; Williams et al. 1986). Especially impressive here is the finding by Northcote and co-workers (1988) of a 37% higher concentration of HDL$_2$-C in elite veteran endurance athletes as compared to age matched sedentary controls.

Attempts to correlate various indices of fitness with HDL-C have produced ambiguous results. Schnabel and Kindermann (1982) have found a significant direct relationship between VO$_2$max and HDL-C and Kuusi and colleagues (1982) detected a positive correlation between HDL$_2$-C and fitness (defined by a standard amount of work achieved on a maximal exercise test). Other investigations, however, have failed to demonstrate correlations between VO$_2$max or exercise performance and HDL-C (Allison et al. 1981; Brownell et al. 1982; Haskell et al. 1980; Stein et al. 1990). One of the reasons for these inconsistencies may be that VO$_2$max is a relatively insensitive index of endurance fitness and of the changes in endurance capacity which often accompany training (Williams and Nute, 1982).

Another approach taken by some investigators has been to relate the quantity of exercise performed to changes in HDL-C concentration. Moore and co-workers (1983) found that distance ran correlated well with HDL-C
concentrations in a group of female distance runners and a study by Cook and colleagues (1986) examining active postal carriers showed a significant relationship between the reported number of miles walked per day and HDL-2-C. Other cross-sectional studies, however, categorising runners into groups according to running mileage, have been unable to demonstrate increased concentrations of HDL-C in the most prolific runners (Higuchi et al. 1989; Watt et al. 1990). In their one year longitudinal study Wood and co-workers (1983) found a dose-response relationship between miles ran and HDL-C although the changes in HDL-C did not start to occur until a threshold of about eight miles of running per week had been reached. Interestingly in this study, those subjects who had the highest initial HDL-C concentrations also ran the most miles during the study. This suggests that a self-selection effect may be important in determining who will gain the most benefit from exercise which could in turn be influenced partly by genetic factors (Despres et al. 1988).

The influence of exercise intensity on lipoprotein concentration remains controversial and has not been researched into as extensively as some other areas. Gaesser and Rich (1984) found no differences in the effects of high (80-85% of VO₂max) or low (45% of VO₂max) intensity exercise on blood lipids over an 18 week training period, neither treatment was effective in elevating HDL-C levels significantly. In contrast, Stein and colleagues (1990) found cycling at 75% of maximal heart rate to be optimal for elevating HDL-C compared to cycling at 65% and 85% of maximal heart rate. A limitation to this study, however, was the fact that the group training at 75% of maximal heart rate had the lowest concentrations of HDL-C to begin with. To add to this confusion Stubbe and associates (1983) found a significant negative correlation between changes in HDL-C and training intensity (expressed as a percentage of maximal pulse) albeit a weak one. There is inadequate information then for the prescription of an optimal training intensity but it is clear that both high intensity exercise i.e. running (Wood et al. 1985) and low intensity exercise such as walking (Hardman and Hudson, 1991) can stimulate increases in HDL-C.
2.5.4 Exercise, triglycerides, chylomicrons and very low density lipoprotein cholesterol

Both cross-sectional and longitudinal studies have usually reported lower values of plasma triglycerides for endurance trained subjects as compared to sedentary controls (Giada et al. 1988; Moore et al. 1983; Northcote et al. 1988; Sady et al. 1988; Thompson et al. 1988; Weintraub et al. 1989). This lower triglyceride concentration is usually mirrored by lower plasma concentrations of VLDL since it is these particles which carry the bulk of triglyceride in the body in the fasted state (Haskell, 1986).

The effect of exercise on chylomicrons has to be studied in the postprandial state since the half-life of chylomicron triglyceride is less than five minutes. After consumption of a high fat meal endurance trained subjects usually exhibit lower levels of chylomicrons than untrained controls (Cohen et al. 1989; Weintraub et al. 1989). This is due to a decrease in chylomicron triglyceride half-life mediated partly by a reduction in the fasting serum triglyceride pool size and partly by a direct effect of chronic exercise on the triglyceride removal system (Cohen et al. 1989).

2.5.5 Exercise and Apoproteins

Relatively few studies have examined the influence of exercise on apoproteins although their measurement has become more common recently. Attention has focused primarily on apoproteins A and B due to their relationships with HDL-C and LDL-C respectively and the hypothesis that apoproteins A-I and B may provide a better indication of CHD risk than either HDL-C or LDL-C (Durrington, 1989).

Cross-sectional studies which have measured apoproteins A-I and A-II usually find increased concentrations in those who are physically active (Herbert et al. 1984; Nagao et al. 1988; Sady et al. 1988; Thompson et al. 1983) although this is not always the case (Giada et al. 1988). Moreover, total inactivity is related to decreased concentrations of Apo A-I (Nikkila et al. 1980). The results from longitudinal studies are much less consistent and although some have shown increases in Apo A-I and/or Apo A-II after a period of training (Kiens et al. 1980; Mendoza et al. 1991; Stubbe et al. 1983) many others have not (Despres et al. 1990; Hespel et al. 1988a; Iltis et al. 1984; Marti et al. 1990;
Thompson et al. 1988; Weintraub et al. 1989; Wood et al. 1983). On the basis of these findings Despres and colleagues (1990) suggest that exercise may lead to "... an increase in HDL content rather than HDL particle number" but this suggestion is not consistent with the findings from cross-sectional studies.

Both observational and longitudinal studies have reported lower levels of Apo B in physically active subjects as compared to control subjects (Despres et al. 1990; Giada et al. 1988) but more frequently Apo B concentrations appear to be unchanged by training (Despres et al. 1988; Iltis et al. 1984; Marti et al. 1990; Weintraub et al. 1989; Wood et al. 1983) although Wood and colleagues (1983) did find a negative correlation between Apo B and miles ran per week in their one year training study. At present there is insufficient information to come to any firm conclusions regarding the effects of physical conditioning on apoproteins and this will remain the case until future well designed studies address this issue.

2.5.6 Mechanisms of influence

The means by which endurance exercise provokes changes in plasma lipoproteins is not fully understood but most explanations propose that the enzyme lipoprotein lipase plays a central role in this process (Katan, 1990; Krauss, 1989; Thompson, 1990). As mentioned in the Section on lipid metabolism, lipoprotein lipase is the rate limiting enzyme which facilitates the removal of triglyceride from chylomicrons and VLDL. In this process cholesterol, protein and phospholipid remnants are transferred to 'nascent' HDL particles which have been secreated from the liver. Taskinen and Nikkila (1981) have demonstrated a positive correlation between the lipoprotein lipase activity of both adipose tissue and skeletal muscle and HDL$_2$-C concentrations. It has been hypothesised, therefore, that an exercise induced increase in lipoprotein lipase activity leads to enhanced triglyceride catabolism and thus augments HDL-C production via the provision of additional substrate for HDL formation.

Considerable evidence exists to support this theory. It has been demonstrated, for example, that the activity of lipoprotein lipase is significantly higher in distance runners than sedentary individuals (Herbert et al. 1984; Williams et al. 1986) and that lipoprotein lipase activity can be increased after a programme of endurance training (Peltonen et al. 1981; Thompson et al. 1988). In addition physically active individuals have reduced concentrations of
triglycerides which are inversely correlated to their increased lipoprotein lipase activity (Sady et al. 1984). Furthermore, endurance trained subjects have an enhanced ability to clear an intravenous infusion of fat which is directly related to both their increased concentrations of HDL-C (and HDL2-C) and their elevated lipoprotein lipase activity (Sady et al. 1986; Sady et al. 1988).

More direct evidence to support the above theory has been provided by two recent well designed studies, one by Kiens and Lithell (1989) and the other by Ruys and co-workers (1989). Keins and Lithell (1989) isolated the quadriceps femoris muscle group in six men and trained one leg by dynamic exercise for eight weeks while the other leg served as a control. After training there was a 70% greater muscle lipoprotein lipase activity as well as a significantly higher formation of HDL-C and HDL2-C in the trained muscle as compared to the untrained muscle. There was also an increase in muscle capillary density in the trained leg which correlated well with the increased lipoprotein lipase activity and provides evidence that greater muscle capillarisation increases the number of binding sites for the lipoprotein lipase enzyme to cleave triglyceride from VLDL. In addition a larger capillary density would imply a longer mean transit time of blood through the muscle thus increasing the potential for fatty acid oxidation as well as the contact time between VLDL and lipoprotein lipase (Kiens and Lithell, 1989).

Ruys and co-workers (1989) examined the peripheral production of HDL-C by measuring the arteriovenous blood flow across the forearm muscle at rest and after 20 minutes of isometric exercise. Subjects were studied twice, once after a fast and once after a high fat meal. Exercise led to an increased production of HDL-C in both conditions but this was much more pronounced in the fed as compared to the fasted condition. By contrast, there was no peripheral production of HDL-C in two subjects with lipoprotein lipase deficiency. These findings give weight to the theory that lipoprotein lipase activity in the muscle capillary bed is at least in part responsible for the exercise related increase in HDL-C and suggest that the supply of chylomicrons and VLDL (and therefore triglyceride) influences the amount of HDL-C formed by lipoprotein lipase. A suggestion which is supported by the high correlation between HDL production and VLDL triglyceride degradation found during exercise by Kiens and Lithell (1989).
Although HDL-C production may be enhanced during acute exercise there is evidence to suggest that the primary effect of chronic exercise is not to increase HDL-C synthesis but to reduce HDL-C degradation. Herbert and associates (1984) using radioiodinated autologous HDL have found that runners do not produce more HDL protein than sedentary subjects but rather catabolise less (the mean biologic half-life of HDL proteins was 6.2 days in the runners compared with 3.8 days in the sedentary men). These findings have been confirmed in the longitudinal training study of Thompson and colleagues (1988) in which the synthetic rates of apoproteins A-I and A-II were not affected but the biological half-life of Apo A-II was increased. The mechanisms by which training might prolong HDL survival are unknown but they may relate to the lipid enrichment of HDL particles both during and after exercise which could theoretically increase the core to surface ratio and therefore retard HDL degradation (Thompson, 1990).

Another possible explanation for the prolonged survival of HDL may be an altered activity of the enzyme hepatic lipase which is thought to be involved in the catabolism of HDL-C. Hepatic lipase activity has been shown to correlate negatively with HDL-C in runners (Sady et al. 1984) and is lower in runners than sedentary men (Marniemi et al. 1980; Williams et al. 1986). Moreover, the activity of hepatic lipase has been found to be negatively correlated with HDL2-C and positively correlated with HDL3-C perhaps indicating that hepatic lipase is involved in the conversion of HDL2-C to HDL3-C as proposed by Nikkila and colleagues (cited in Haskell, 1986). The reduced level of hepatic lipase activity associated with exercise training may thus be an explanation for the higher levels of HDL2-C often reported in those who are physically fit (Kuusi et al. 1982).

The LCAT enzyme has also been implicated in the processes leading to elevated HDL-C with endurance exercise. As mentioned this enzyme catalyses the transfer of fatty acids in the plasma from lecithin to cholesterol (forming cholesterol esters) during the production of HDL-C. An enhanced activity of LCAT would therefore suggest an increased production of HDL-C. However, although some studies have demonstrated an increase in the activity of LCAT after both acute (Frey et al. 1991) and chronic exercise (Marniemi et al. 1982) other studies have failed to show any changes in LCAT activity with training (Iltis et al. 1984; Williams et al. 1990b) and thus the LCAT hypothesis remains controversial.
The means by which exercise leads to reduced levels of LDL-C are very poorly understood. Several mechanisms have been proposed acting separately or in combination with each other. These include a reduced synthesis of VLDL (the precursor for LDL-C) by the liver or an impaired conversion of VLDL remnants to LDL-C; an increased uptake of LDL-C by peripheral cells mediated through enhanced LDL receptor activity or an increase in the removal of LDL-C by the liver. To date, very little direct evidence is available to substantiate any of these theories (Haskell, 1986).

2.5.7 Exercise, weight loss and changes in lipoproteins

There is considerable debate as to whether or not the changes in lipoproteins which occur during exercise operate independently from the reductions in body weight, or more especially the reductions in body fat, which are often concurrent with training. Attempts to correlate the percentage of body fat with HDL-C in observational studies have produced ambiguous results with some investigations revealing an inverse correlation between these two variables (Thompson et al. 1983) while others show them to be unrelated (Sady et al. 1984). Findings from longitudinal investigations have generally been more consistent, however, and although exercise results in an increase in HDL-C without changing body fat in some studies (e.g. Hardman and Hudson, 1991; Stein et al. 1990; Thompson et al. 1988) more often elevations in HDL-C occur concomitantly with reductions in body weight/body fat (Despres et al. 1990; Hespel et al. 1988b; Marti et al. 1990; Sasaki et al. 1987; Wood et al. 1985).

Williams and co-workers (1983) investigated the question of whether or not weight loss is responsible for the exercise induced increase in HDL-C in their one year training study. Although the changes in HDL-C were not significantly different between the exercise and control group, within the exercise group one year changes in HDL-C were strongly correlated with body weight changes. Interestingly, however, comparison between the exercise and control groups by regression analysis revealed that equivalent amounts of weight change corresponded to a significantly greater change in HDL-C in the exercisers than the controls. Williams and colleagues (1983) concluded that the metabolic consequences of an exercise induced weight loss are different from the consequences of weight change in the sedentary state. This seems plausible since in the first instance weight loss is associated with an increased energy turnover while in the second case energy turnover is decreased.
However, further studies by this group have provided conflicting results. For example, in one study fat loss caused by either diet or exercise was equally effective in elevating HDL-C concentrations (Wood et al. 1988). In a more recent study, however, a dietary induced weight loss was not effective in elevating HDL-C concentration whereas weight loss elicited by a combination of diet and exercise did significantly increase HDL-C concentration (Wood et al. 1991).

Williams (1990) has postulated that weight loss is an essential factor in the exercise provoked increase in HDL-C and that level of HDL-C is more strongly related to the amount of weight lost than the actual leanness achieved. To support his argument Williams (1990) cites the following evidence. Firstly, although it is true that lipoprotein lipase activity is increased in runners it is the adipose tissue lipoprotein lipase activity which predominates accounting for 79% of the whole body lipoprotein lipase activity (Nikkila et al. 1978). Therefore, greater triglyceride hydrolysis is likely to occur in adipose tissue than skeletal muscle. Moreover, Savard and co-workers (1985) have shown that adipose tissue lipoprotein lipase activity is negatively correlated with fat cell diameter in runners and Tremblay and colleagues (1984) found that runners who were fat before running had smaller fat cells than those who were lean initially. Therefore, those people who lose the most weight during an exercise programme are likely to have the smallest fat cells, the highest adipose tissue lipoprotein lipase activity and thus the greatest concentrations of HDL-C (Williams, 1990).

Despite the evidence cited above it is not certain that weight loss is the means by which exercise exerts a beneficial effect on lipoproteins. Changes in body composition are an integral part of exercise conditioning and the extent of these changes may be a reflection of the effectiveness of the training programme while being merely coincidental to the changes in lipoprotein concentration. It has already been stated, for example, that at least some of the increase in HDL-C found with exercise is due to training induced adaptations in skeletal muscle tissue (Kiens and Lithell, 1989). It is likely, however, that both exercise and weight loss have independent effects which are most pronounced when operating in conjunction with one another. This proposal is supported by Tran and Weltman's (1985) 'meta-analysis' of 95 studies which measured changes in lipoprotein levels in response to exercise training. Their findings indicated that although changes in total cholesterol and LDL-C did
occur in the absence of any effect on body weight, reductions were greatest when exercise training was combined with weight loss.

Before concluding this discussion it is worth mentioning that there are several advantages to a weight loss caused through exercise as compared to a weight loss elicited by diet alone. Firstly, an exercise induced weight loss is not associated with the decrease in lean body mass which often accompanies a weight loss induced by diet (Wood et al. 1988). Secondly, an exercise induced weight loss has been shown to cause a greater increase in HDL$_2$-C than a weight loss induced by diet alone (Williams et al. 1990a). Finally, there is evidence that changes in body fat are more easily achieved and maintained when exercise forms part of a weight loss programme (Martin and Dubbert, 1982).

2.5.8 Conclusion

Sportsmen and women performing large amounts of endurance exercise exhibit plasma lipoprotein profiles which denote a low risk of developing CHD. When previously inactive individuals undertake a programme of regular endurance type exercise they often demonstrate changes in their lipoprotein profiles which resemble more closely the lipoprotein profiles of elite, endurance trained, sportsmen and women. These changes, however, are not as great as those seen in elite athletes which may be due to the fact that the volume of training performed by such athletes is much greater than that which most people can, or are willing to, undertake.

In all probability the amount of exercise required to provoke changes in lipoprotein profiles will vary tremendously depending on subjects' initial lipoprotein concentrations and perhaps also their initial level of fitness. As a generalisation, however, an exercise level equivalent to 10 miles of jogging per week appears to be enough to elicit changes in most previously sedentary people (Superko, 1991). This may be related to the amount of energy expended in exercise rather than the actual intensity of exercise since chronic low intensity activity is also associated with improved lipoprotein profiles (Cook et al. 1986).
The most consistent effects of exercise are related to alterations in the concentration of HDL-C rather than total cholesterol or LDL-C and elevations of up to 27% in HDL-C have been reported (Hardman and Hudson, 1991). Changes of this nature could have a profound influence on CHD mortality since a 1% increase in HDL-C is associated with a 1.5 to 2% reduction in CHD rates (Rifkind, 1990).
2.6 Exercise and other selected coronary heart disease risk factors

2.6.1 Blood pressure

Elevated blood pressure is one of the major risk factors for CHD as demonstrated by the Framingham study (Kannel et al. 1980). Furthermore, Paffenbarger and co-workers (1986) found that hypertensive men had nearly twice the risk of death (from all causes) of normotensive men. The link between exercise and blood pressure has been clearly established in epidemiological studies such as that of Paffenbarger and colleagues (1983) which showed that men who did not engage in vigorous sports play were at a 35% higher risk of suffering from hypertension than those who did. Moreover, Blair and co-workers (1984) have demonstrated that men and women with low levels of cardiorespiratory fitness have a 52% greater risk of developing hypertension than those with high levels of cardiorespiratory fitness.

Since the findings from epidemiological studies cannot rule out a self-selection effect a number of longitudinal studies have been conducted to assess the potential of exercise to independently influence blood pressure in hypertensive subjects (systolic blood pressure >140 mm Hg and/or diastolic blood pressure >90 mm Hg). In a review of these studies Hagberg and Seals (1986) concluded that exercise training is capable of eliciting approximately 10 mm Hg reductions in both systolic and diastolic blood pressure although they point out that many studies suffer from design deficiencies, the major one being the absence of a control group. Tipton has also reviewed the effectiveness of exercise as a means of lowering blood pressure and he concluded that training results in 5 to 25 mm Hg reductions in systolic blood pressure and 3 to 15 mm Hg reductions in diastolic blood pressure. Moreover, Tipton noted that the most profound reductions in blood pressure occur in older subjects who have followed prolonged training programmes (Tipton, 1991; Tipton, 1984).

Recently the ability of exercise to influence hypertension has been confirmed in many training studies varying in length from ten weeks to nine months all of which used a control group (Hagberg et al. 1989; Kelemen et al. 1990; Martin et al. 1990; Sasaki et al. 1989; Sommers et al. 1991; Tanabe et al. 1988). In most of these studies there were no changes in body weight/body fat or other
confounding variables supporting an independent blood pressure lowering effect of endurance exercise. Moreover, on the basis of findings such as these increased physical activity is now recommended by the World Hypertension League (1991) as a therapeutic treatment for hypertensive patients.

As might be expected the influence of exercise on blood pressure in normotensive adults is not as pronounced as it is in hypertensive subjects. Nevertheless, observational studies usually reveal a difference in blood pressure of up to 5 mm Hg between the most and least physically active subjects (Fagard, 1985). Furthermore, Tipton (1984) in his extensive review concluded that endurance training is likely to lower resting blood pressure slightly in normotensive subjects if their initial pressures are in excess of 125/85 mm Hg. Recently, studies by Gilders and colleagues (1989) and Verity and colleagues (1990) have failed to demonstrate any significant reduction in the blood pressure of normotensive subjects who completed four months and five years of endurance training respectively. Since blood pressure was initially below 125/85 mm Hg in these subjects, however, the conclusions of Tipton (1984) would seem to be confirmed.

The most beneficial type of activity for reducing blood pressure appears to be continuous exercise involving large muscle groups such as in running, swimming and cycling although there is some evidence that weight training may also be beneficial (Hagberg and Seals, 1986). The frequency of exercise required to reduce blood pressure has been studied and cycling performed seven days per week has been found to produce more dramatic drops in blood pressure than cycling performed only three days per week (Nelson et al. 1986). However, as Jennings and co-workers (1989) point out about three-quarters of the antihypertensive effects of exercise in this study occurred at the lower level of activity and this supported their conclusion that the effects of exercise on blood pressure are maximal with fairly moderate levels of activity.

It seems that the intensity of exercise does not need to be severe to influence blood pressure. Roman and colleagues (1981) have reported similar reductions in blood pressure after one year of either high (80% of VO₂max) or low (50% of VO₂max) intensity training despite the fact that the high intensity training increased the subjects VO₂max by twice as much as the low intensity training. More recently, Hagberg co-workers (1989) have shown that exercise performed at 53% of VO₂max can produce greater reductions in blood
pressure than exercise performed at 73% of VO2max. In this study VO2max increased by 28% in the high intensity exercise group but did not change in the low intensity group. Findings such as these have led Gordon and colleagues (1990) in a recent review to conclude that changes in blood pressure do not parallel increases in maximal oxygen uptake and that moderate intensity exercise may be more effective in controlling hypertension than high intensity exercise.

The mechanisms by which exercise training might lead to a reduction in blood pressure are not well understood. However, since mean blood pressure is the product of cardiac output and total peripheral resistance (Astrand and Rodahl, 1986) one or both of these two variables must be reduced if blood pressure is to be lowered. Resting heart rate is often decreased after a period of training and therefore it has been proposed that this underlies the changes in blood pressure accompanying exercise because if stroke volume and total peripheral resistance are unchanged or increase less than the reduction in heart rate, blood pressure must be lowered (Hagberg and Seals, 1986).

Another training adaptation which could be responsible for the antihypertensive effect of exercise is the often reported decrease in resting concentrations of plasma noradrenaline which would cause a reduction in sympathetic nervous system activity and thus a decrease in the total peripheral resistance (Jennings et al. 1989). Since the hypertensive state is characterised in most cases by systemic arteriolar constriction with an increase in systemic vascular resistance while cardiac output remains unchanged (Gorlin, 1991) this theory seems more credible. However, it is possible that the mechanisms underlying a reduction of blood pressure in the hypertensive state are different from those which underlie a reduction of blood pressure in the normotensive state.

Other postulated mechanisms for an exercise induced decrease in blood pressure include a resetting of baroreceptors, an altered distribution of blood volume and changes in the renin-angiotensin axis although evidence to support any of these theories is limited (Gordon et al. 1990). Moreover, it has also been shown that a single bout of aerobic exercise leads to reductions in blood pressure for one to three hours following activity in hypertensive subjects (Hagberg et al. 1987; Kaufman et al. 1987). Therefore, it is possible that the reduction in blood pressure associated with regular participation in
endurance exercise is due to the cumulative effects of single exercise bouts rather than long term adaptations to exercise training.

In conclusion, exercise can be of benefit in reducing blood pressure in hypertensive patients although it should not be regarded as a panacea. Exercise is also capable of eliciting small reductions in blood pressure in normotensive subjects and can be considered an effective means of offsetting the elevations in blood pressure which occur during the ageing process (Astrand and Rodahl, 1986). Moreover, the significance of these reductions should not be underestimated since Paffenbarger and colleagues (1986) have found that amongst normotensive persons mortality rates are lower for those whose systolic blood pressure is below 130 mm Hg than it is for those with higher readings. The mechanisms by which exercise leads to reductions in blood pressure are not well understood and evidence to support the current theories is limited. Finally, it seems that the frequency and intensity of exercise need only be moderate to exert maximal effects on blood pressure although information regarding the ability of low level exercise programmes such as walking to influence blood pressure is limited.

2.6.2 Fibrinogen

Fibrinogen is a glycoprotein which plays an essential role in haemostasis. This complex process is initiated when the collagen fibres of a damaged blood vessel become exposed thus promoting the formation of a platelet plug. At the same time the coagulation cascade is initiated by the activation of factor XII (intrinsic clotting pathway) and/or factor VII (extrinsic clotting pathway) which culminates, after many intermediate steps, in the conversion of soluble fibrinogen circulating in the blood into insoluble fibrin. This conversion is facilitated by the enzymatic action of thrombin and serves to support and reinforce the platelet plug by solidifying the blood in the area of the wound. Clot formation is halted via the action of prostacyclin (also termed prostaglandin I₂, or PGI₂), protein C and antithrombin C. After the damaged vessel has been repaired the clot is dissolved when plasminogen, stimulated by activators such as t-PA (tissue plasminogen activator), is converted to plasmin which digests fibrin in a process termed fibrinolysis (Vander, Sherman and Luciano, 1990; Witt, 1985).
The fact that fibrinogen may be implicated in the development of CHD has been revealed in many epidemiological studies which were published in the 1980s. The first of these was the Northwick Park Heart Study (Meade et al. 1980) which examined 1511 white men aged between 40 and 64 over a six year period and found that high levels of fibrinogen at entry to the study were strongly related to the incidence of CHD. Furthermore, this relationship was even stronger than the relationship between cholesterol and CHD. The findings of the Northwick Park Heart Study were subsequently confirmed by the Goteborg Study (Wilhelmsen et al. 1984), the Leigh Study (Stone and Thorpe, 1985) and the Framingham Study (Kannel et al. 1987) all of which found fibrinogen to be a positive risk factor for CHD. Kannel and colleagues (1987) concluded that "the impact of fibrinogen value... on cardiovascular disease (is) comparable with the major risk factors... (and) should be added to the cardiovascular risk profile". More recent findings from angiographic (Handa et al. 1989), case control (Qizilbash et al. 1991) and epidemiological studies (Yarnell et al. 1991) support this conclusion.

Both environmental and genetic factors are probably responsible for causing elevated plasma fibrinogen concentrations (Ernst, 1990; Meade, 1987). Smoking is the leading environmental determinant of fibrinogen concentrations as demonstrated by the Framingham Study where there was a dose-dependent relationship between the number of cigarettes smoked per day and the age adjusted fibrinogen level in both men and women (Kannel et al. 1987). Indeed it is possible that a large portion of the association between smoking and CHD could be accounted for by the increased fibrinogen concentrations found in smokers (Meade, 1987).

The means by which elevated fibrinogen levels may predispose individuals to CHD are complex and many mechanisms have been proposed (see Cook and Ubben, 1990). An obvious possibility is the increased chance of clot formation in the coronary vessels associated with a hypercoagulable state. However, fibrinogen may also be involved in the early stages of atherosclerotic plaque formation since fibrinogen degradation products have been found in atherosclerotic lesions and the amount of fibrin deposition correlates linearly with plasma fibrinogen concentrations. Fibrin in turn may aid in the migration of cholesterol to atherosclerotic plaques by providing an absorptive surface for LDL-C accumulation. Handa and colleagues (1989) reported a strong association between plasma fibrinogen and serum
cholesterol concentration and it is possible that fibrinogen and LDL-C act synergistically in the development of atherosclerosis. Increased levels of fibrinogen in vitro have also been shown to elevate measures of platelet aggregability. Moreover, aside from erythrocyte concentration, fibrinogen is the major determinant of blood viscosity and increased blood viscosity characterises many cardiovascular disorders (Cook and Ubben, 1990; Ernst, 1990).

There is conflicting evidence as to the interaction between exercise, coagulation and fibrinolysis (see Bourey and Santoro, 1988). This is almost certainly related to the complex nature of blood haemostasis and the many factors involved in both coagulation and fibrinolysis. Acute exercise has been shown to cause both an increase (Arai et al. 1990) and decrease (Osterud et al. 1989) in plasma fibrinogen levels. The significance of any changes in fibrinogen concentration after acute exercise cannot be considered in isolation, however, since both coagulability and fibrinolysis are enhanced as a result of acute exercise (Arai et al. 1990). These changes in coagulability and fibrinolysis after acute exercise are related to increases in factor VIII activity and t-PA activity respectively (Bourey and Santoro, 1988). Information regarding the long term effects of exercise on fibrinogen concentrations is limited but it has been found that endurance trained individuals have lower concentrations of fibrinogen and lower plasma viscosity values than sedentary controls (Ernst, 1990).

In summary then, elevated fibrinogen concentrations promote a state of hypercoagulability and thus may contribute to the pathological and clinical onset of CHD. Cross-sectional studies have revealed lower concentrations of fibrinogen in endurance trained individuals and acute exercise has been shown to influence both coagulation and fibrinolysis. However, the effect of chronic exercise on fibrinogen concentration in previously sedentary subjects has yet to be determined.

2.6.3 Pulmonary Function

The relationship between pulmonary function and CHD has been examined in a number of large epidemiological studies including the Framingham Study (Kannel et al. 1983), the Seven Countries Study (Keys et al. 1972), the Honolulu Heart Programme (Marcus et al. 1989), the Gothenburg Study in
Sweden (Persson et al. 1986) and the British Regional Heart Study (Cook and Shaper, 1988). All of these studies have shown that impairments in forced vital capacity (FVC) and/or forced expiratory volume in one second (FEV\textsubscript{1}) are associated with an increased risk of developing CHD. Moreover, impaired pulmonary function is also predictive of an elevated risk for myocardial infarction (Friedman et al. 1976), subsequent development of hypertension (Selby et al. 1990; Sparrow et al. 1988) and overall mortality (Beaty et al. 1982; Krzyzanowski and Wysocki, 1986).

There is no clear cut explanation for the link between pulmonary function and cardiovascular morbidity and mortality but a number of theories have been advanced. It has been suggested, for example, that a decreased vital capacity is simply reflective of occult or undiagnosed heart disease. However, Weiss (1991) argues that this is unlikely because in the above studies there is no other initial evidence for coronary disease in the subjects who subsequently go on to develop it. Another hypothesis is that cigarette smoking, which is a common risk factor for both decreased lung function and CHD, explains the link between the two. However, although this appears to be true in some studies (Keys et al. 1972; Marcus et al. 1989), more often it does not seem to be the case (Ashley et al. 1975; Beaty et al. 1982; Cook and Shaper, 1988; Friedman et al. 1976; Krzyzanowski and Wysocki, 1986; Persson et al. 1986).

Other explanations for the link between reduced lung function and CHD centre on the associations between lung function and various established risk factors for CHD including exercise. Kannel and colleagues (1983), for example, suggest that FVC may be a measure of "overall vigour and general health". This suggestion is based on the fact that the correlation between FVC and handgrip strength in the Framingham study was stronger than the correlations between FVC and the other cardiovascular risk factors which they examined. More recently, Higgins and colleagues (1991), examining data from the CARDIA Study (Coronary Artery Risk Development in Young Adults) found that height adjusted FEV\textsubscript{1} (FEV\textsubscript{1}/Ht\textsuperscript{2}) was correlated positively with a history of strenuous physical activity and with the duration of exercise on a treadmill. Height adjusted FEV\textsubscript{1} also correlated positively with HDL-C and negatively with smoking, triglycerides and skinfold thicknesses in this study. The authors concluded that the above associations may well explain the link between pulmonary function and heart disease.
If physical activity does partly explain the link between CHD and pulmonary function it is not necessarily a causative link. Pulmonary function is predominantly determined by factors such as age, sex and body size, especially height. Measures of lung function have not been shown to be of any great value in predicting fitness or exercise performance in healthy individuals (McArdle et al. 1981). Moreover, elite athletes often have lung volumes which are normal for their age and height (Kaufmann et al. 1974; Ness et al. 1974) and although larger than normal lung volumes have been reported in some groups of physically active people (Newman et al. 1961) these are generally attributed to differences in genetic endowment (Keys et al. 1972; McArdle et al. 1981).

In conclusion, reduced lung function is now an established risk factor for heart disease. The reasons for this link are not clear but are most likely related to the association between lung function and other cardiovascular risk factors including perhaps exercise. Whether these associations are coincidental to the development of heart disease or are causatively involved remains to be determined.
2.7 Adapations to endurance training

2.7.1 Introduction

In previous Sections of this Chapter the epidemiological evidence linking exercise and CHD has been discussed. The literature relating to the influence of exercise on lipid and lipoprotein metabolism has been reviewed and the effects of exercise on other selected risk factors for CHD including blood pressure, fibrinogen and pulmonary function has been investigated. In each case there has been some implication that regular exercise may be of value in reducing CHD risk. Moreover, in each instance, the evidence cited concerns primarily endurance type exercise involving large muscle groups in sustained periods of physical activity. In the final Section of this Chapter the physiological and metabolic adaptations which result from a period of endurance training are discussed. This information could provide clues as to the possible mechanisms, direct or indirect, by which exercise may influence CHD.

Endurance training causes many adaptations to take place within the body which collectively result in an enhanced ability to perform both maximal and submaximal exercise. \( \text{VO}_2\text{max} \) is the most universally applied measurement which has been employed to assess training induced improvements in fitness and an increase in \( \text{VO}_2\text{max} \) after training has been reported in numerous studies. Perhaps the most explicit demonstration of the effects of physical activity on \( \text{VO}_2\text{max} \) is provided by the classical bed rest study which was devised by Saltin and colleagues (1968). In this study \( \text{VO}_2\text{max} \) was reduced in all five subjects after a three week period of total inactivity. Subsequent to this, post bed rest \( \text{VO}_2\text{max} \) values were enhanced by up to 126% during a 50 day training programme. More often, however, the increase in \( \text{VO}_2\text{max} \) after a period of training is in the order of 5 to 20% (Astrand and Rodahl, 1986).

An elevation in \( \text{VO}_2\text{max} \) will contribute to an enhanced capacity for maximal exercise since there is a strong linear relationship between oxygen uptake and work rate. In addition to increased oxygen uptakes, however, trained individuals are able to exercise at higher relative intensities i.e. higher percentages of their individual \( \text{VO}_2\text{max} \) and can maintain a given percentage of their \( \text{VO}_2\text{max} \) for longer than their less active counterparts (Sjodin and Svedenhag, 1985). This ability is reflected in a reduced blood lactate
concentration at the same relative and absolute submaximal exercise intensity in trained persons (Hurley et al. 1984; Seals et al. 1984b). Also heart rate is lower in trained subjects as compared to sedentary subjects at any given absolute submaximal exercise intensity (Astrand and Rodahl, 1986). As a result of these changes there is a reduced disturbance in the homeostasis of the body at any given submaximal exercise intensity in the trained individual as compared to the untrained individual and thus the 'stressfulness' of exercise is decreased.

The adaptations responsible for eliciting these changes may be broadly classified into cardiovascular and metabolic adaptations. The extent of these adaptations will obviously depend on many factors including the mode, duration, frequency and intensity of exercise, the period of time over which the training programme is conducted, the initial level of fitness possessed by the subject and probably also genetic factors.

2.7.2 Cardiovascular adaptations to endurance training

Training induced adaptations in the cardiovascular system will result in an improvement in oxygen transporting capacity and therefore enhance the delivery of oxygen to the working muscles. There is strong evidence that cardiovascular rather than respiratory or metabolic factors limit oxygen uptake (see Saltin and Rowell, 1980) during whole body exercise and therefore the cardiovascular adaptations which occur as a result of training can be quantified to some extent by measuring VO2max. According to the Fick principle, VO2max is the product of cardiac output multiplied by the arteriovenous oxygen difference ((a-v) O2 difference) and thus for VO2max to increase one or both of these factors must be enhanced.

Since maximal (a-v) O2 difference changes little after the early stages of training, cardiac output is the most important factor dictating changes in VO2max (Brooks and Fahey, 1984) and thus there is a strong relationship between these two variables. Elite endurance athletes are able to generate cardiac outputs of around 40 l.min⁻¹ compared to 20 l.min⁻¹ in sedentary individuals (McArdle et al. 1981). Indeed Astrand and Rodahl (1986) calculated that a cardiac output of around 48.4 l.min⁻¹ was possible in one individual who had a VO2max of 7.4 l.min⁻¹. "Both an intensive training and superb natural endowment (would) contribute to... (this) remarkable circulatory
capacity" (Astrand and Rodahl, 1986). Maximal heart rate is unaffected or slightly lowered by endurance training thus the larger cardiac outputs exhibited by elite athletes result exclusively from an increase in maximal stroke volume (McArdle et al. 1981). Both myocardial and extramyocardial adaptations combine to increase stroke volume.

The effects of exercise on heart size have been examined using echocardiography. Peronnet and co-workers (1981) reviewed a number of these studies and they concluded that endurance trained individuals generally have larger heart volumes than their sedentary counterparts while similar although not as profound results have been attained from longitudinal training studies. These greater heart volumes are primarily due to increases in the left ventricular end diastolic volume since wall thickness does not change appreciably with endurance training. This is in contrast to the myocardial adaptations occurring as a result of strength training which are characterised by an increase in wall thickness without any change in ventricular volume (Peronnet et al, 1981). Whether or not there is any change in the inherent contractile ability of the heart remains controversial but seems unlikely (Blomqvist and Saltin, 1983).

The mechanisms responsible for a post-training increase in the end diastolic volume have been reviewed by Blomqvist and Saltin (1983). One possibility is that the enlarged end diastolic volume could simply be related to an increased ventricular dilation caused by an exercise induced bradycardia. At least some of the increase in end diastolic volume, however, appears to be due to a true increase in muscle mass since training has been shown to result in an elevated heart-weight/body-weight ratio. Moreover, the cardiac hypertrophy consonant with training is widespread since left atrial and right ventricular dimensions are also consistently increased in subjects with left ventricular hypertrophy. Accompanying the larger cardiac dimensions which result from physical conditioning, experimental evidence in animals has revealed that exercise training results in an increase in the size of the coronary vascular bed, involving both capillaries and larger vessels. This will obviously improve the oxygen supply to the myocardium thus facilitating its enhanced pumping ability (Blomqvist and Saltin, 1983).
In addition to a larger heart size other factors are also related to the elevated stroke volume including an increased preload and/or a decreased afterload. Endurance training causes an increase in plasma volume and to a lesser extent red blood cell volume which together result in approximately an eight per cent increase in total blood volume (Lamb, 1984). It is possible that this increase in blood volume could enhance the venous return and thus lead to an increase in the end diastolic volume (preload). This in turn would stretch the myocardium and cause a more powerful ejection stroke in accordance with Starling's law of the heart. As a result the normal stroke volume would be discharged as well as the extra blood which entered the ventricular and stretched the myocardium. There is evidence to support this theory from studies in which infusions of 'plasma expanders' or whole blood have resulted in an increase in both stroke volume and cardiac output (Kanstrup and Ekblom, 1982; Spriet et al. 1980).

A decrease in the systemic resistance (also termed total peripheral resistance) to ventricular filling (afterload) must also be involved in the increased stroke volume otherwise the effects of an enhanced heart size and end diastolic volume would largely be negated. The reduced afterload may be caused to some extent by a more extensive capillarisation of skeletal muscle but more important quantitatively is a reduction in the arteriolar resistance. This occurs predominantly in the arterioles supplying the working skeletal muscle and is caused by a regulatory vasodilation (Vander, Sherman and Luciano, 1990). The mechanisms behind this regulation are poorly understood but it is likely that vasodilation is mediated through skeletal muscle receptors and/or changes in the metabolic state of the muscle.

The increase in stroke volume with training is mirrored by a decrease in heart rate both at rest and during submaximal exercise. Thus, cardiac output remains unchanged at a given submaximal exercise intensity after training. The reduced heart rate in the trained individual may be a consequence of an elevated vagal activity and a reduced sympathetic activity or it could simply be caused by the increase in stroke volume. Whatever the mechanism a decreased heart rate will reduce the energy requirement and oxygen demand of the heart and therefore improve its economy (Astrand and Rodahl, 1986).
As alluded to earlier, endurance training enhances the capillary density of skeletal muscle. This was clearly demonstrated by Ingjer (1979) who found that the number of capillaries per muscle fibre increased from 1.39 pre-training to 1.79 post-training. This amounted to a 28.8% increase over a 24 week training period. An enhanced capillary density will have the effect of increasing the capillary blood volume and thus the mean transit time of blood through the capillaries (since the determinant of mean transit time is capillary blood volume divided by the rate of blood flow through the capillaries). In addition to the increase in mean transit time there will also be a greater capillary surface area and taken together these two factors will promote a more complete exchange of gases, substrate and metabolites between the blood and muscles thus allowing a high cardiac output to be maintained without compromising the (a-v) $O_2$ difference.

In summary then the cardiovascular adaptations to exercise result in an increase in maximal stroke volume and therefore maximal cardiac output. Heart rate is reduced at a given submaximal exercise intensity thus reducing myocardial oxygen demand and improving the economy of the heart muscle. There is also an increase in the capillary density of skeletal muscle and a decrease in total peripheral resistance which facilitates blood flow to the working muscles and therefore oxygen delivery. Collectively these factors will result in an increase in oxygen uptake and the ability to perform maximal exercise.

2.7.3 Metabolic adaptations to endurance training

The alterations in skeletal muscle metabolism which occur as a consequence of training have been reviewed extensively by Saltin and Gollnick (1983). Major changes include an increase in the number and size of mitochondria and an enhanced activity of various enzymes in the mitochondria including those involved in the citric acid cycle and electron transfer chain and the enzyme beta-hydro-acyl-CoA-dehydrogenase which is involved in the beta-oxidation of free fatty acids. As a result of these changes the oxidative capacity of skeletal muscle is improved and there is a greater contribution of fat to the total energy requirement at a given submaximal exercise intensity (Matoba and Gollnick, 1984).
Unlike the training induced adaptations in the cardiovascular system changes in the metabolic capacity of skeletal muscle do not appear to cause an elevation in VO$_2$max but rather serve to enhance endurance performance during submaximal exercise (Saltin and Rowell, 1980). This is due largely to the increased concentrations of the enzymes involved in fat metabolism which have the effect of increasing the reaction velocity (Vmax) of fat metabolism at a given substrate concentration. The fatty acid flux through the oxidative pathways is thus enhanced and the Embden-Meyerhof pathway inhibited (Gollnick and Saltin, 1982). The result is a greater reliance on fat for energy supply at a given submaximal exercise intensity which reduces the contribution of muscle glycogen. Since there is a close correspondence between the termination of exercise due to fatigue and the depletion of glycogen within the working muscles during endurance activity, a sparing of muscle glycogen will have the effect of prolonging exercise performance (Coyle and Coggan, 1984).

The precise mechanism by which an increase in the oxidative capacity of skeletal muscle might facilitate submaximal exercise performance remains somewhat perplexing however. Although blood lactate concentration is often reduced in the trained individual at a given submaximal exercise intensity, suggesting an increase in the oxidative contribution to energy provision, oxygen uptake is usually similar in the trained and untrained state at any given submaximal work rate (Holloszy and Coyle, 1984). This anomaly is poorly addressed in literature describing the metabolic adaptations to endurance exercise but some authors have attempted to explain it.

Matoba and Gollnick (1984), for example, argue that it is a misconception to consider the appearance of lactate as being synonymous with an insufficiency of oxygen since lactate can be produced in skeletal muscle when there is not a shortage of oxygen. They suggest that the cause of this situation may be a poor control over glycogenolysis. An increase in mitochondrial size, number and enzyme activity after training then may "function as a great sink for the translocation of adenosine diphosphate... out of the cytosol and into the mitochondria". This would reduce the concentration of adenosine diphosphate and perhaps also adenosine monophosphate in the cytosol therefore inhibiting the activation of glycogenolysis. The end result would be a reduced production of pyruvate and a reduced conversion of pyruvate to lactate. Holloszy and Coyle (1984) also subscribe to this theory and conclude that
exercise at a given work rate "requires a smaller percentage of the muscles' maximum respiratory capacity (after training) and therefore results in less disturbance in homeostasis".

Brooks and Fahey (1984) propose an additional mechanism to explain the significance of an increase in the size and number of mitochondria. They state that the utilisation of oxygen in the mitochondria is associated with a liberation of free radicals which accumulate causing mitochondrial damage and ultimately fatigue. A greater size and number of mitochondria, however, will reduce the impact of a given accumulation of free radicals thus delaying the onset of fatigue. Although these hypotheses require clarification there is no doubt that the trained individual is able to sustain a given submaximal work rate with an increased utilisation of fat (as evidenced by a lower respiratory exchange ratio), a reduced degradation of muscle glycogen and a lower concentration of lactate in the blood. Together these factors enhance submaximal exercise capacity.

2.7.4 Blood lactate concentration as an indicator of adaptations to endurance training

As mentioned above VO$_2$max has been the traditional measurement used to assess the changes in the fitness of an individual after a period of training. It should be apparent from the preceding discussion, however, that changes in VO$_2$max will not fully reflect the adaptations which occur with endurance training. This has been demonstrated effectively by Davies and colleagues (1981) who found only a 14% elevation in VO$_2$max but a 100% increase in the mitochondrial content of muscle and a 403% increase in endurance capacity after a ten week training programme in rats. Studies using human subjects have also found a disparity between changes in VO$_2$max and changes in endurance capacity after training (Hardman, 1982; Williams and Nute, 1982). The reason for these findings is that VO$_2$max is to a large extent genetically predetermined (Klissouras et al. 1973) and thus can only be enhanced to a limited extent under normal circumstances. In contrast, however, skeletal muscle has a profound ability to adapt when additional stresses are placed upon it and the resultant metabolic changes which occur may have a significant impact on endurance performance without altering VO$_2$max.
In an attempt to make a more complete assessment of the adaptations consequent to endurance training many physiologists are now measuring the concentration of lactate in the blood during submaximal exercise. The rational for this procedure is the belief that changes in blood lactate concentration after training are reflective of alterations in the oxidative capacity of skeletal muscle and in particular improvements in mitochondrial efficiency. In short then, blood lactate concentration during submaximal exercise is thought to be indicative of muscle respiratory (mitochondrial) activity. Moreover, submaximal blood lactate concentration has been shown to correlate well with endurance performance (Sjodin and Jacobs, 1981; Williams and Nute, 1983) and may provide a better indication of improvements in endurance capacity than \( \text{VO}_2\text{max per se} \) (Williams and Nute, 1986).

The inter-relationships between exercise and lactate metabolism are discussed thoroughly by Brooks and Fahey (1984) and also other authors (Astrand and Rodahl, 1986; Lamb, 1984; McArdle et al. 1981). Although a detailed discussion of this subject is beyond the scope of this review a few points seem worth highlighting. Firstly, at physiological pH lactic acid dissociates virtually completely to hydrogen and lactate ions and thus the terms lactic acid and lactate are used synonymously. Lactic acid is a product of glycogenolysis and glycolysis, processes which involve the chemical breakdown of glycogen and glucose respectively to pyruvic acid (the terms glycogenolysis and glycolysis are used interchangeably). Glycolysis can occur both in the presence and in the absence of oxygen. When oxygen supply is plentiful most of the pyruvic acid derived from glycolysis enters the mitochondria and is ultimately oxidised in the citric acid cycle. When oxygen supply is limited, however, pyruvic acid is converted to lactic acid via the enzyme lactate dehydrogenase. Eventually, the lactic acid formed in the muscle passes into the blood.

Anaerobic glycolysis provides a rapid means of supplementing the energy provided from aerobic metabolism and is particularly important during maximal or near maximal exercise but the resulting increase in lactate and thus hydrogen ion concentration is associated with fatigue in some cases. Mechanisms do exist for the removal of lactate from the blood and this has been studied in animals using radioisotopes (Brooks et al. 1973; Brooks and Gaesser, 1980). These studies have revealed that the endpoints of lactate metabolism are diverse but the majority of lactate is either oxidised in the citric
acid cycle or serves as a precursor for glucose and glycogen synthesis in the liver. This process is accomplished via the cori cycle and is termed gluconeogenesis. During heavy exercise, however, the capacity for lactate removal via gluconeogenesis is limited to a great extent by a reduced hepatic blood flow.

Lactic acid is a dynamic metabolite then which is constantly being produced and removed even at rest. During light exercise the production of lactic acid may increase several fold without causing significant changes in the concentration of lactic acid in the blood due to matching increases in the rate of lactate removal. In heavy exercise blood lactate concentration increases to a greater extent but provided the removal rate is proportionally elevated a new steady state will eventually be attained. This indicates that lactate removal is concentration dependent with increased concentrations stimulating enhanced removal rates. If increases in lactate concentration are unchecked, however, then production outstrips removal and fatigue will eventually follow. One of the effects of training is that it enhances the removal of lactate from the blood as demonstrated by Donovan and Brooks (1983).

Before concluding this discussion it is worth noting some reservations about the terminology frequently used in this area. Brooks and Fahey (1984) state that the term anaerobic glycolysis as applied to lactic acid production is misleading because it suggests that a lack of oxygen is responsible for the formation of lactate. They contend that this is not the case but that lactate formation depends on mitochondrial activity. Moreover, the mitochondria are in active competition with lactate dehydrogenase, the terminal enzyme of glycolysis, for pyruvate and because of the large equilibrium constant ($K_{eq}$) of this enzyme some lactate will always be formed regardless of the presence of oxygen. It is of interest to note that endurance training may lead to a decrease in the activity of lactate dehydrogenase (Brooks and Fahey, 1984).

2.7.5 Adaptations to walking exercise

Most of the studies investigating the adaptations to training have examined relatively intense forms of exercise such as running. Moreover, the subjects studied have been predominantly young adult males. However, although the literature is comparatively sparse some studies have examined the training
effects of low intensity forms of exercise such as walking using older groups of people.

Pollock and colleagues (1971), for example, examined the changes in VO$_2$max in a group of 16 middle-aged men who walked for 40 minutes four times a week over a 20 week period. Walking progressed from 2.5 to 3.25 miles per session and walking speed increased from 4.3 to 4.7 miles per hour over the 20 week period. The average exercise heart rate increased from 132 beats per minute at the beginning of the study to 146 beats per minute by the end and VO$_2$max was enhanced by 28%. This relatively large increase in VO$_2$max was attributed to the poor initial “fitness level” (VO$_2$max) of the subjects. Supporting this conclusion Porcari and co-workers (1987) have found that the potential of fast walking to elicit a training stimulus (exercise intensity >70% of maximal heart rate) is negatively related to VO$_2$max. Moreover, Pollock and colleagues (1975) subsequently repeated their study using a younger (presumably fitter) group of subjects and found that the increase in VO$_2$max was only 11%.

Since the studies of Pollock and colleagues (1971) and Pollock and colleagues (1975) a number of other investigations have shown that walking training can increase VO$_2$max in middle-aged and older individuals. In a study by Seals and co-workers (1984a), for example, VO$_2$max increased by 11% in a group of men and women (average age 63 years) after six months of low-intensity training which included 20-30 minutes of walking (heart rate <120 beats/min) at least three times per week (average 4.6 times per week). Furthermore, Jette and associates (1988) examined the physiological responses of a group of previously sedentary men and women (age range 35-53 years old) to a 12 week training programme. Subjects were required to walk at a pace which elicited 60% of VO$_2$max for 30 minutes three times a week. This programme increased the VO$_2$max of both the males and the females by 13.3% and 16.5% respectively. More recently still Steinhaus and co-workers (1990) have demonstrated that a four month training programme involving walking and jogging can produce beneficial changes in the VO$_2$max of previously sedentary men and women aged between 55 and 70 years old.

Very little information is available regarding the mechanisms behind a walking induced improvement in the VO$_2$max of older individuals but Seals and colleagues (1984a) did address this question. Cardiac output was measured
during submaximal treadmill walking via a non-invasive CO₂ rebreathing method and since heart rate was also monitored they were able to calculate the submaximal stroke volume. Maximal cardiac output was then ascertained by multiplying the stroke volume during submaximal exercise by the heart rate recorded at VO₂max. The rational for this being that stroke volume is maximal at submaximal exercise intensities (>40% of VO₂max) in upright exercise. Finally, maximal a-v O₂ difference was determined by dividing VO₂max by maximal cardiac output. The results revealed a small but significant six per cent increase in the estimated maximal stroke volume after six months of low intensity training which produced a non-significant six per cent increase in maximal cardiac output. The a-v O₂ difference was also six per cent higher after training and since this was significant the authors concluded that the elevated VO₂max was primarily a consequence of the increase in maximal a-v O₂ difference.

Aside from increases in VO₂max walking training has also been shown to provoke beneficial changes in the responses to submaximal exercise. For example, heart rates are reduced at a given absolute exercise intensity after training (Hudson et al. 1988; Jette et al. 1988). Moreover, Hardman and colleagues (1989b) have demonstrated lower blood lactate concentrations during both walking and stepping exercise after a six month training programme and Seals and co-workers (1984b) found that blood lactate concentration was reduced at the same absolute and relative exercise intensities after the six months of low intensity training completed by their subjects. In contrast to these findings Jette and co-workers (1988) did not find any significant change in the submaximal blood lactate concentration of their subjects after 12 weeks of training despite a significant increase in VO₂max.

In the study by Seals and colleagues (1984b) respiratory exchange ratio (R) values were also lower at the same absolute work rate after training indicating a shift in substrate utilisation toward an increased oxidation of fat. The R values were similar, however, at the exercise intensities which produced a blood lactate concentration of 2.5 mmol.l⁻¹ before and after low intensity training despite the higher energy expenditures at this blood lactate concentration after training (18.8 ml.kg⁻¹min⁻¹ before training verses 22.0 ml.kg⁻¹min⁻¹ after training). Therefore, the calculated oxidation rate of carbohydrate was 16% higher after low intensity training compared with before training at the exercise intensity which elicited a blood lactate
concentration of 2.5 mmol.l⁻¹. The authors concluded that a similar lactate concentration despite a greater carbohydrate oxidation after training suggested that a smaller proportion of the pyruvate formed during glycolysis was converted to lactate and a greater proportion was channelled into the mitochondria. This in turn suggested an increase in the oxidative capacity of the skeletal muscles i.e. an enhanced ability of the mitochondria within the muscles to compete with lactate dehydrogenase for pyruvate.

In summary then, evidence exists to show that low intensity exercise such as walking can result in cardiovascular and metabolic adaptations which are similar to those elicited by training programmes of a higher intensity. Moreover, although the extent of the adaptations provoked by low intensity exercise may not be as great as those resulting from high intensity exercise, low intensity training may be more beneficial for health, especially in older populations, because of its reduced potential to cause injury. This has been clearly demonstrated by Seals and co-workers (1984a) who found that the incidence of orthopaedic injuries was much more common and of a greater severity during high intensity training than during low intensity training.
3 GENERAL METHODS

3.1 Informed consent

Prior to the start of the study the subjects were made fully aware of all of the procedures which they would have to undertake and any potential risks which they might incur. All subjects were then asked to sign a statement of informed consent (Appendix 1).

3.2 Oxygen uptake and carbon dioxide production

Oxygen uptake and carbon dioxide production during exercise were determined from samples of expired air collected in 150 litre capacity Douglas bags (Harvard Equipment Ltd.). During the collection subjects wore a nose clip (Harvard Equipment Ltd.) and a snorkel type mouthpiece (Harvard Equipment Ltd.). The mouthpiece was fitted to a lightweight two-way respiratory valve (Jakeman and Davies, 1979) which in turn was attached to a 1.5 metre section of wide bore (30 mm diameter) lightweight tubing (Falconia: Baxter, Woodhouse and Taylor). The tubing terminated in a two-way tap (Harvard Equipment Ltd.) which was used to open and close the Douglas bag.

The oxygen content (%) of expired air was measured using a paramagnetic oxygen analyser (Taylor: Servomex Model 570A) while carbon dioxide content (%) was determined using an infra-red carbon dioxide analyser (Lira: Mines Safety Appliances Ltd. Model 3030). Both analysers were calibrated before each exercise test using certified reference gases (CryoService Ltd. Worcester, U.K.). All reference gases were in turn calibrated against a gold standard reference gas (CryoService Ltd. Worcester, U.K.). This gold standard reference gas was used throughout all of the testing reported in this thesis to ensure consistency. The volume of expired air withdrawn for analysis was measured using a flow meter.

Once the oxygen and carbon dioxide analysis had been completed the volume of air remaining in each Douglas bag was determined by evacuation (Moulinex vacuum pump 237) through a Harvard dry gas meter which had been calibrated against a 600 litre Tissot spirometer (Collins Ltd.). The air temperature was measured during evacuation by a thermistor placed in the air
outlet pipe of the dry gas meter. The thermister was linked to a thermometer (Edale: type 2984, Model C).

Finally, the measured gas volumes were corrected to standard temperature and pressure for a dry gas (STPD) and the volume of inspired air (VI) was then derived using the Haldane transformation. After this the volume of oxygen consumed (VO2) and carbon dioxide produced (VCO2) were calculated (Appendix 2).

3.3 Exercise tests

Four different exercise tests were employed in the study, three involved walking on a motorised treadmill (Marquette electronics inc.) which was calibrated before and during the study (Appendix 3) while one took place on the University athletics track. Each subject was familiarised with treadmill walking before any tests were undertaken.

3.3.1 Submaximal, incremental treadmill test

The purpose of this test was to determine the relationship between oxygen uptake and work rate during submaximal exercise for each individual. This information was then used to predict each subject's VO2max. The test was sixteen minutes long and divided into four, four minute exercise periods. The speed of the treadmill remained constant throughout the test at either 1.34 m.s⁻¹ or 1.56 m.s⁻¹ (3.0 or 3.5 mph) while the treadmill incline was set at 0 or 3% to begin with and subsequently elevated by 3% at the end of each four minute exercise period. The speed and incline used for the treadmill test were determined by a subjective assessment of each individual's capability. Douglas bag samples of expired air were collected for the last minute of each four minute exercise period and heart rate was monitored continuously during the test via an electrocardiograph (Rigel Cardiac Monitor 302. Rigel Research, U.K. Ltd.) and three chest electrodes (3M U.K. Ltd. Type 2255). The subject's perceived rate of exertion (PRE) was also measured at the end of each stage of the test using the Borg scale (Borg, 1973).
The data gained from this test were used to predict VO$_2$max (ml.kg$^{-1}$ min$^{-1}$) via a method outlined by Maritz and colleagues (1961). Firstly, the average heart rates attained during the last minute of each of the four stages of the test were used as independent (X) variables and matched against the corresponding oxygen uptakes (dependent or Y variables) to create a simple linear regression equation. Then maximum heart rate was predicted via Astrand's (1960) equation of 210 - (0.65 x age) and used as an independent variable to predict the oxygen uptake at maximal heart rate. This value was taken to be VO$_2$max (Figure 3.1).

\[ y = 15.787 + 0.299 \times x \]
\[ r = 1.000; r^2 = 0.999 \]

**Figure 3.1** Prediction of maximal oxygen uptake from submaximal heart rate and oxygen uptake data using simple linear regression.

Once VO$_2$max had been predicted a second linear regression equation was calculated, this time using the oxygen uptakes attained during each stage of the test as the independent variables and the treadmill inclines (%) at which these oxygen uptakes were elicited as the dependent variables. The oxygen consumptions which would be attained if the subject was working at 50%, 60%, 70% and 80% of VO$_2$max were then calculated (i.e. 0.5 x VO$_2$max = oxygen consumption at 50% of VO$_2$max etc.) and used in the regression equation to predict the treadmill inclines necessary to elicit these oxygen consumptions (Figure 3.2). These inclines were subsequently incorporated.
into two further treadmill tests which were thus 'individualised' for each subject as described below.

Figure 3.2 Calculation of the treadmill inclines required to elicit 50%, 60%, 70% and 80% of predicted VO$_2$max.

There are inherent problems in estimating VO$_2$max from submaximal exercise data and predictions of maximal heart rate (for a discussion see Astrand and Rodahl, 1986: p.372-380). Errors are obviously likely to occur if predictions are made from regression equations when data are extrapolated outside the range from which they were generated. Astrand and Rodahl (1986) also note that the standard deviation for maximal heart rate within an age group is ±10 b.min$^{-1}$, thus VO$_2$max is bound to be underestimated in some individuals and overestimated in others. Moreover, emotional factors, nervousness and apprehension may affect heart rate during light and moderate intensity exercise thus resulting in erroneous predictions of VO$_2$max.

In contrast to heart rate, oxygen uptake is usually very constant at a given submaximal rate of exercise. This is true even after a period of training although small changes may take place. In view of this it should be recognised that changes in predicted VO$_2$max within an individual reflect changes in heart rate for a given oxygen uptake. Although these changes in heart rate may
result from training i.e. they may be due to an increase in stoke volume, there is also the chance that they merely reflect reductions in the subject's level of anxiety. If the latter is true then this will give the false impression that the subject's VO2max has changed when this may not in fact be the case.

Despite the above observations it was felt that the prediction of VO2max was justified in the present study because it would have been inappropriate to conduct maximal exercise tests. This was due to the nature of the subject group (i.e. previously sedentary, middle-aged men) and the fact that medical supervision was not readily available while testing was in progress. Furthermore, the primary purpose of predicting VO2max was to ensure that the subsequent submaximal exercise tests were set at an appropriate level for each individual. This should still have been the case even if there were small errors in prediction.

3.3.2 Grade-lactate treadmill test

The protocol for this test was similar to that of the first treadmill test but this time the treadmill grades generated from the regression equation described above were used. Thus, each subject was exercising at approximately 50% of VO2max during the first four minutes of the test, 60% of VO2max in the second four minutes, 70% of VO2max during the third four minutes and 80% of VO2max for the last four minutes of the test. Once again heart rate and PRE were recorded during the last minute of each stage of the test while collections of expired air were taking place. In addition duplicate 20 μl thumb prick samples of capillary blood were collected at the end of each stage of the test and later analysed for lactate.

From the results of this test the oxygen uptake and thus the %VO2max attained by each subject at the reference blood lactate concentrations of 2 and 4 mmol.l⁻¹ could be ascertained. This was accomplished by plotting oxygen uptake (ml.kg⁻¹.min⁻¹) against blood lactate concentration and interpolating across from the 2 and 4 mmol.l⁻¹ points on the blood lactate axis as shown in Figure 3.3.
Figure 3.3 Determination of the oxygen uptakes attained at the reference blood lactate concentrations of 2 and 4 mmol.l\(^{-1}\).

3.3.3 Endurance walk

This test was conducted to examine each subject’s physiological and metabolic responses to steady state exercise. The test was 20 minutes in length and was conducted at a constant incline calculated to elicit 70% of \( VO_2 \text{max} \) throughout. Expired air samples were collected between minutes four and five, nine and ten, 14 and 15 and 19 and 20 during the test at which times heart rate and PRE were also being recorded. Thumb prick samples of capillary blood were taken at five minute intervals and later analysed for lactate.

3.3.4 1600 m Track walk

The purpose of this test was to assess brisk walking pace and intensity (as percentage of maximum heart rate). The subjects walked exactly four laps of the track and thus covered slightly less than one mile (i.e. 1600 m as opposed to 1609 m). Instructions to the subjects were as follows: "walk at a pace which is brisk but not maximal and which you could maintain for about 30 to 45 minutes if necessary". Heart rate was monitored at 15 second intervals throughout this test by means of a short range telemetry system (PE 3000
Sports-tester, Sweden) which also recorded the time taken to complete the walk.

3.4 Blood Sampling

Thumb prick samples of capillary blood were collected both at rest and during exercise for the measurement of lactate concentration. Before samples were taken at rest the hand was immersed in warm water to increase superficial blood flow and thus arterialise the capillary blood. The skin was punctured using an Auto-clix automatic lancet (Boehringer Mannheim, U.K. Ltd.) and two 20 μl samples of blood were collected into micro-pipettes (Acupette Pipettes; Scientific Industries Ltd.). The samples were immediately deproteinised in 200 μl of 2.5% perchloric acid and subsequently centrifuged (Eppendorf centrifuge: model 5414) at 12,000 rpm for 30 seconds before being placed in a freezer (-70 °C) where they remained until analysis.

Twenty millilitre volumes of blood were obtained from the subjects by venepuncture after they had completed a 12 hour overnight fast. The fast was required because the concentration of triglycerides in the blood is affected by the proximity of meals (Durrington, 1990). Samples were usually collected between 8:00 and 10:00 am to standardise for any daily variation but exceptions were made occasionally if this time was inconvenient. Subjects remained in the sitting position while the sample was being drawn unless they had a tendency to faint in which case samples were collected while the subject laid on a couch. In most cases the sample was drawn within one minute of venepuncture. In an attempt to limit error due to biological variation two samples were collected from each subject not more than five days apart and the mean value of these two samples was adopted as the best estimate of the true value.

Once the blood sample had been obtained, a 2 ml portion of this blood was poured into a plastic citrated tube (Sarstedt 9NC/2) and placed into a refrigerator (4 °C) while the remaining blood was dispensed into 10 ml plastic tubes (Sarstedt Z/10). From these tubes two 20 μl samples were collected into micro-pipettes and immediately diluted in 5 ml of Drabkin's solution from which haemoglobin concentration was determined later in the day. Three further samples of blood were collected into heparinized micro-haematocrit tubes (Scientific Industries Ltd.) and subsequently centrifuged for 15 minutes.
(Hawksley micro-haematocrit centrifuge). From these tubes haematocrit was determined.

The blood left in the 10 ml plastic tubes was allowed to clot for 45 minutes before being centrifuged (Burkard Koolspin set at 4 °C) along with the 2 ml sample at 6,000 rpm for 15 minutes. The citrated plasma and most of the serum were then removed from the tubes using pasteur pipettes (Scientific Industries Ltd.) and divided into separate 1 ml plastic aliquots which were immediately frozen at -70 °C. A small (2-3 ml) portion of serum was not frozen, however, but placed into plastic tubes and stored in a refrigerator. Sometime within the next five days this sample underwent precipitation to remove LDL-C and VLDL-C and in some cases HDL₂-C also. The supernatant resulting from this procedure was dispensed in 1 ml aliquots and stored with the others at -70 °C.

3.5 Blood biochemistry

Full details of the blood biochemistry are included in the Appendices while a brief overview is given here. Blood lactate was measured on a fluorimeter (Locarte, London. Model 8-9) using a modification of the enzymatic method described by Maughan (1982) (Appendix 4). Haemoglobin concentration (Appendix 5) was determined photometrically (Eppendorf photometer 1101 M) by a cyanmethemoglobin method using a commercially available test kit (Boehringer Mannheim, U.K. Ltd.). Haematocrit was ascertained using a micro-haematocrit reader (Hawksley). All of these procedures took place in the biochemistry laboratory at Loughborough University.

Total cholesterol concentration (Appendix 6) was determined using an enzymatic kit (Boehringer Mannheim, U.K. Ltd.). To isolate HDL-C a manganese chloride/sodium heparin precipitation was performed and this was followed by a second precipitation using dextran sulphate if HDL₃-C concentration was required (Appendix 7). The concentrations of HDL-C and HDL₃-C were determined using an enzymatic kit (Appendix 8). The concentration of HDL₂-C was calculated as HDL-C minus HDL₃-C. Measurements of triglyceride (Appendix 9) were also made using an enzymatic kit (Boehringer Mannheim, U.K. Ltd.) and were not corrected for free glycerol. Low density lipoprotein cholesterol and VLDL-C were estimated in
chylomicron-free serum using the Friedewald formula (Friedewald et al. 1972) (Appendix 10).

Apoproteins A-I and B (Appendix 11) were determined by immunoprecipitin analysis using commercially available kits (Atlantic Antibodies, Inc. U.S.A.). An enzyme-immunoassay was employed to determine Lp(a) concentration again using a commercially available kit (Appendix 12). The sensitivity of this assay was such that values below 5 mg.dl$^{-1}$ could not be detected but since it has now been established that Lp(a) is present in virtually all individuals (Durrington, 1989) values of 1 mg.dl$^{-1}$ were assigned to those subjects whose Lp(a) concentration was undetectable.

The coefficient of variation for some of the above assays is included in Appendix 13. To ensure accuracy and precision were being maintained, quality control sera were used throughout (Precinorm L, Boehringer Mannheim, U.K. Ltd.).

Except in the case of the storage study (see below) all of the lipid and lipoprotein assays were completed using an automated analyser (Cobas Mira) in the Department of Chemical Pathology at the Royal Free Hospital School of Medicine, London. The aliquots of serum were transported from Loughborough University to the Royal Free Hospital in an ice box which was filled with dry ice and polystyrene. Samples were stored until the end of the study before being transported thus enabling all of the serum from one individual to be analysed in the same batch. Furthermore, each batch included samples from both walkers and controls thus reducing the likelihood of finding systematic differences between the two groups as a result of experimental procedures.

Lipid and lipoprotein analysis during the storage study was performed manually in the biochemistry laboratory at Loughborough University. A photometer (Eppendorf photometer 1101 M) was used to read the absorbance of the samples.

Fibrinogen concentration was determined by a coagulation assay (Appendix 14). Analysis was performed in the Department of Human Sciences at Loughborough University by Miss Jayshree Savania. Only one sample was analysed for each subject at each observation point during the brisk walking
study. Since fibrinogen is an "acute phase protein" it should be borne in mind that individual elevations in plasma fibrinogen concentration may occur when infections are present (Ernst, 1990).

3.6 Blood pressure

Arterial blood pressure was determined using a random zero sphygmomanometer (Hawksley) according to the procedures described by Elliott and Stamler (1988). Subjects were seated for five minutes with their legs uncrossed before measurements were initiated using the right arm. Usually two measurements were made and the mean value taken to represent the true value. In the case of poor agreement between measurements further determinations of blood pressure were made.

3.7 Spirometry

A dry spirometer (Vitalograph Ltd.) was used in the assessment of various indices of lung function including forced vital capacity (FVC), forced expiratory volume in one second (FEV₁) and the percentage of FVC exhaled in one second (FEV₁/FVC x 100). Subjects were fully familiarised with the equipment prior to testing. During the tests subjects assumed a standing position and wore a nose clip. They were first instructed to inhale as deeply as possible, and then after sealing their lips around the mouthpiece, they were encouraged to exhale as forcibly and completely as possible. Exhalation usually continued for six seconds. Measurements were repeated after a short rest interval if the subject or experimenter felt that a maximal effort was not achieved.

3.8 Dietary analysis

The seven day weighed food inventory technique was used to monitor subjects diets. The methods used for this analysis are described by Brooke-Wavell (1992).

3.9 Anthropometry and body composition

The procedures used for measuring height, body mass, various circumferences and skinfold thicknesses and body density (hydrostatic weighing) are described by Brooke-Wavell (1992). From these measurements
the amount and distribution of body fat in each subject were determined. Body mass was also measured before each treadmill test using a beam balance (Avery). In this situation subjects remained lightly clothed but removed their shoes.

3.10 Statistical analysis

Most of the statistical procedures used in this thesis are outlined by Cohen and Holliday (1982). One way analysis of variance was used to test the difference between many means obtained by repeated measurement of one group. Two way analysis of variance for repeated measures was used to test for differences when repeated measurements were made on two groups. This enabled an evaluation of whether or not the responses of the two groups differed from each other (interaction effect) and also indicated any changes within each group over time (repeated measures). Relationships between variables were examined using the Pearson Product Moment correlation coefficient.

When the assumptions underlying parametric statistics were not met non-parametric methods were employed as follows. The Mann Whitney U test was used to detect differences between two sets of scores originating from different groups. The Wilcoxon matched pairs test was employed to test for significance when repeated measurements were made on the same group and the strength of a relationship between two variables was assessed via the Spearman rank order correlation coefficient.

Due to the skewed nature of the triglyceride and Lp(a) data a logarithmic transformation (using natural logarithms) was employed to normalise the data. The antilog of the mean of the log data was then used to represent the geometric mean of the group and a 95% confidence interval on the log scale was antilogged to give a confidence interval on the original scale. The procedures for this form of data treatment are described by Gardner and Altman (1989).

Values reported in this thesis are mean plus or minus the standard error of the mean (SEM) unless otherwise stated and the five per cent confidence level has been adopted throughout although in many cases the degree of significance is much higher.
3.11 Storage study

3.11.1 Introduction

Lipid and lipoprotein analysis should ideally be performed on fresh serum samples within 24 hours of collection, however, this is often unrealistic and samples are usually frozen and assayed at a later date. The effects of storage on lipid and lipoprotein concentrations are variable depending on, amongst other things, the length of time over which the sample is stored, the temperature at which the sample is stored and the type of lipid or lipoprotein being measured. The concentrations of both total cholesterol (Pini et al. 1990) and HDL-C (Kohlmeier and Schlierf, 1982) have been shown to change significantly over time when stored at -20 °C and although storage at -70 °C seems to provide a more stable environment (Nanjee and Miller, 1990) significant changes have been found even at this temperature (Bachorik, 1982). Therefore, a storage study was initiated, the aim of which was to establish whether or not a systematic variation occurs in the concentrations of total cholesterol and/or HDL-C from serum samples frozen for various periods of time. A further aim of this study was to assess the extent to which storage affects the measurement of HDL3-C since this proved problematic during the walking study i.e. in many cases HDL2-C could not be precipitated.

3.11.2 Methods

Twenty millilitre samples of venous blood were collected from 16 fasted subjects on the same morning. Measurements of serum total cholesterol, HDL-C and HDL3-C were then made on a portion of the fresh serum from each subject. The remainder of the serum was dealt with in the following way for each subject. Four 50 µl samples of serum were pipetted into aliquots and frozen at -70 °C from which total cholesterol was later determined. Four 1 ml samples of serum were pipetted into plastic tubes and frozen at -70 °C for later precipitation and determination of HDL3-C. Four 1 ml samples of serum were precipitated immediately to remove LDL-C and VLDL-C and then frozen at -70 °C. From these samples HDL-C was later to be determined.

Thus, for each subject a total of 12 samples were now stored in the freezer, four for total cholesterol, four for HDL-C and four for HDL3-C. Each set of four samples was labelled 'A', 'B', 'C' and 'D'. On the following day (i.e. 24 hours
after collection) each of the subject's 'A' samples (i.e. one for total cholesterol, one for HDL-C and one for HDL3-C) was thawed and analysed. The purpose of this was to assess the effects of the process of freezing/thawing per se. This routine was repeated for the 'B' samples three months later, for the 'C' samples six months later and for the 'D' samples nine months later. The values gained from samples 'A', 'B', 'C' and 'D' were now compared with those gained from the initial analysis of fresh serum in order to determine the effects of storage. The methods used for the determination of total cholesterol, HDL-C, and HDL-C subfractions were those described in Sections 3.4 and 3.5.

An alternative to the above protocol would have been to follow the approach used in the storage study of Nanjee and Miller (1990). In this study each fresh sample was divided into two portions. One of these portions was subsequently frozen at -70 °C while the other was frozen in liquid nitrogen. Both portions of the sample were analysed together after a given period of storage. The sample frozen in liquid nitrogen was then used as reference with which its paired sample, frozen at -70 °C, was compared. This method assumes stability of samples stored in liquid nitrogen but has the advantage that all samples are analysed in the same batch thus preventing error due to between batch variation. In the method described earlier this is not the case since the fresh (reference) samples are analysed separately from the frozen samples. In the event, however, the liquid nitrogen method was not used because it would have created logistical problems and increased the expense of the study.

3.11.3 Results

Table 3.1 below displays the values obtained for each lipid and lipoprotein measured during the storage study in addition to giving calculated values for HDL2-C and the ratio of total cholesterol to HDL-C. The percentage change in each of these quantities during the study is shown in Table 3.2 while Table 3.3 gives the correlation coefficients obtained when comparing the base-line values of each lipid and lipoprotein with those subsequently attained. Changes over time were assessed using one way analysis of variance for repeated measures. Significant changes are indicated on Table 3.2.
<table>
<thead>
<tr>
<th></th>
<th>Base line</th>
<th>1 day</th>
<th>3 months</th>
<th>6 months</th>
<th>9 months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TC</strong></td>
<td>4.47 ± 0.14</td>
<td>4.29 ± 0.14</td>
<td>4.34 ± 0.14</td>
<td>4.57 ± 0.14</td>
<td>4.37 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>3.21 - 5.40</td>
<td>3.04 - 5.12</td>
<td>3.23 - 5.33</td>
<td>3.40 - 5.39</td>
<td>2.96 - 5.27</td>
</tr>
<tr>
<td><strong>HDL-C</strong></td>
<td>1.50 ± 0.10</td>
<td>1.35 ± 0.10</td>
<td>1.50 ± 0.10</td>
<td>1.23 ± 0.10</td>
<td>1.48 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>0.93 - 2.56</td>
<td>0.85 - 2.32</td>
<td>1.01 - 2.52</td>
<td>0.89 - 2.15</td>
<td>0.98 - 2.69</td>
</tr>
<tr>
<td><strong>HDL3-C</strong></td>
<td>0.92 ± 0.04</td>
<td>0.98 ± 0.04</td>
<td>0.67 ± 0.05</td>
<td>0.88 ± 0.04</td>
<td>0.91 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>0.67 - 1.38</td>
<td>0.74 - 1.37</td>
<td>0.39 - 1.19</td>
<td>0.68 - 1.30</td>
<td>0.75 - 1.30</td>
</tr>
<tr>
<td><strong>HDL2-C</strong></td>
<td>0.62 ± 0.08</td>
<td>0.40 ± 0.08</td>
<td>0.86 ± 0.07</td>
<td>0.36 ± 0.07</td>
<td>0.60 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>0.25 - 1.45</td>
<td>0.02 - 1.22</td>
<td>0.51 - 1.62</td>
<td>-0.09 - 1.12</td>
<td>0.23 - 1.66</td>
</tr>
<tr>
<td><strong>TC/HDL-C</strong></td>
<td>3.16 ± 0.23</td>
<td>3.37 ± 0.24</td>
<td>3.01 ± 0.18</td>
<td>3.96 ± 0.23</td>
<td>3.13 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>1.97 - 5.20</td>
<td>2.07 - 5.53</td>
<td>1.97 - 4.50</td>
<td>2.37 - 5.46</td>
<td>1.86 - 4.84</td>
</tr>
</tbody>
</table>

Table 3.1 Values for total cholesterol (TC), HDL-C, HDL2-C, HDL3-C and the TC/HDL-C ratio during the nine month storage study. The top figures in each row are mean ± SEM while the bottom values give the range.
<table>
<thead>
<tr>
<th></th>
<th>1 day</th>
<th>3 months</th>
<th>6 months</th>
<th>9 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>-4.0*</td>
<td>-2.9*</td>
<td>2.2</td>
<td>-2.2</td>
</tr>
<tr>
<td>HDL-C</td>
<td>-10.0*</td>
<td>0.0</td>
<td>-18.0*</td>
<td>-1.3</td>
</tr>
<tr>
<td>HDL3-C</td>
<td>6.5*</td>
<td>-27.7*</td>
<td>-4.4</td>
<td>-1.1</td>
</tr>
<tr>
<td>HDL2-C</td>
<td>-35.5*</td>
<td>38.7*</td>
<td>41.9*</td>
<td>-3.2</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>6.7</td>
<td>-4.8</td>
<td>25.3*</td>
<td>-1.0</td>
</tr>
</tbody>
</table>

Table 3.2 The percentage change from base-line values of the various lipids and lipoproteins assessed during the storage study. * Significantly different from base line.
<table>
<thead>
<tr>
<th>Base line</th>
<th>1 day</th>
<th>3 months</th>
<th>6 months</th>
<th>9 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>0.994</td>
<td>0.961</td>
<td>0.990</td>
<td>0.977</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.944</td>
<td>0.981</td>
<td>0.856</td>
<td>0.961</td>
</tr>
<tr>
<td>HDL₃-C</td>
<td>0.900</td>
<td>0.952</td>
<td>0.970</td>
<td>0.902</td>
</tr>
<tr>
<td>HDL₂-C</td>
<td>0.834</td>
<td>0.984</td>
<td>0.750</td>
<td>0.925</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>0.957</td>
<td>0.977</td>
<td>0.807</td>
<td>0.969</td>
</tr>
</tbody>
</table>

Table 3.3 Pearson Product Moment correlation coefficients obtained from the storage study when comparing base-line lipid and lipoprotein concentrations with concentrations attained after one day, three months, six months and nine months.
3.11.4 Discussion

The changes in total cholesterol were all small (Table 3.2), the greatest change being a decrease of around 4% after one day. Although total cholesterol concentrations at three and nine months were also lower than base-line values the difference was less than 3% while the mean cholesterol concentration after six months was 2.2% higher than base-line values. These findings contrast with those of Pini and colleagues (1990) who found progressive increases (up to 15%) in the concentration of cholesterol from samples stored over just a 14 day period, however, the storage temperature in this study was only -20 °C. Although the decreases found in the present study after one day and three months were significant they are probably simply a reflection of the between batch variation in the cholesterol assay. Nanjee and Miller (1990) note that between batch variation may be caused by many factors including changes in ambient assay conditions and the use of different reagent batches both of which occurred in the present study.

There were significant decreases in HDL-C concentration of 10% and 18% after one day and six months respectively. The correlation coefficient comparing base-line HDL-C concentrations with those at six months is also rather poor. Previous studies have observed both increases and decreases as great as 13% in the concentration of HDL-C with storage (see Nanjee and Miller, 1990 for references) but these changes occur after prolonged periods and not after just one day. As far as the 18% decrement after 6 months is concerned this is greater than other changes reported in the literature. However, the mean values obtained at three and nine months are unchanged from base-line values and the correlation coefficients are comparable with those attained in the study by Kohlmeier and Schlierf (1982) though not as strong as those obtained by Demacker and Jansen (1983). Thus, there would not appear to be a systematic variation in the concentration of HDL-C over time but the changes after one day and six months are perplexing.

Aside from the 27.7% decrease after three months the changes in HDL₃-C are not too great although the reductions after one day and three months were both significant. Other studies have shown a significant variation in the concentration of HDL₃-C with storage including that by Jung and associates (1986) who found a 35% increase in HDL₃-C concentration after four days of storage at a temperature of -20 °C. Smaller but still significant changes have
also been observed by Khan and Elkeles (1984) after a three month period of storage at a temperature of -20 °C. Nanjee and Miller (1990), however, in their study comparing samples stored in liquid nitrogen with those stored at -70 °C did not find any deterioration in HDL3-C concentration.

As with the total HDL-C samples discussed above it is difficult to ascertain whether the changes in HDL3-C concentration with storage are the direct result of sample deterioration or are due to some other factor. It was mentioned earlier that difficulty was experienced in the measurement of HDL3-C during the walking study and therefore one of the reasons for undertaking this storage study was to try and ascertain whether or not the length of time over which serum is stored negatively affects the precipitation of HDL2-C. From the results attained this does not appear to be the case since the greatest changes in HDL3-C concentration occurred at the beginning rather than the end of the study.

An examination of the the values for HDL2-C is also unsupportive of the idea that the length of serum storage interferes with the precipitation procedure. The variation in HDL2-C concentration throughout the storage study was greater than the variation in either HDL-C or HDL3-C (Tables 3.1 and 3.2). This would be expected because HDL2-C is a derived value and therefore reflects the variation in both HDL-C and HDL3-C. As with the other lipoproteins, however, there was no systematic variation in HDL2-C which suggests that the problems experienced in the measurement of HDL3-C were related primarily to methodological factors and/or the freezing process per se rather than being due to the actual length of storage.

3.11.5 Conclusion

Although some significant changes were found during the storage study when comparing base-line values with those subsequently attained, none of the lipids or lipoproteins showed a systematic variation over time. However, the changes in HDL-C subfractions, especially the HDL2-C subfraction, were highly variable and suggest that values gained for these lipoproteins may be unreliable. Furthermore, on the basis of the evidence from this investigation it would appear that the main cause of the variation found in HDL-C subfractions was related to the methodology used, i.e. the precipitation procedure, rather than being due to a true sample deterioration caused by storage.
3.12 Pilot study

3.12.1 Introduction

The predominant methods for assessing endurance training status were referred to in the Section regarding training adaptations in Chapter Two. In this discussion it was mentioned that VO2max is inadequate as a complete descriptor of training status due to its strong genetic component and the fact that it is not a sensitive indicator of the peripheral adaptations which occur as a consequence of training. A further limitation as far as the proposed walking study was concerned was the fact that direct determination of VO2max was contraindicated in the subject group under observation. With these factors in mind it was felt that the assessment of changes in blood lactate concentration during submaximal exercise might be a more suitable method for discerning the improvements in exercise capacity likely to occur with training.

Having decided to utilise the blood lactate concentration during submaximal exercise as an index of endurance it was necessary to validate this procedure before embarking on the brisk walking study. This was due to the fact that most of the studies examining the relationship between blood lactate concentration and endurance have used running as the mode of exercise and employed trained subjects. Therefore, a pilot study was initiated to examine this relationship during walking exercise. This study had two main aims. Firstly, to see how strongly the relative exercise intensity (%VO2max) attained at a reference blood lactate concentration relates to endurance performance (determined by a walk to exhaustion at 80% of VO2max). Secondly, to examine whether a rapid loss of metabolic (blood lactate concentration) or cardiovascular (heart rate) homeostasis during an endurance test is predictive of subsequent performance time. In the endurance walk used in the brisk walking study subjects could not be exercised to exhaustion for safety reasons. Therefore, the findings from this pilot study would be important for the interpretation of the endurance walk data collected in the brisk walking study.

This investigation was undertaken jointly with a third year undergraduate student who submitted a dissertation on the findings in partial fulfilment of her degree requirements (Wallington, 1989).
3.12.2 Methods

A group of 11 female students from Loughborough University agreed to take part in the study after a detailed explanation of the procedures involved. Young female subjects were chosen since it was thought that their physical capacity would best resemble that of middle-aged males and yet still permit the assessment of VO$_2$max and endurance capacity directly without great risk of endangering their health. The group was heterogeneous in nature as regards participation in physical activity and contained recreational runners, games players and sedentary individuals. During the study subjects were asked to complete four exercise tests as described below (two of the subjects did not manage to complete all four tests). The equipment used for these tests was identical to that described in Section 3.3. Table 3.4 displays the physical characteristics of the subjects participating in the study. Of particular note is large variation in body mass within the group.

<table>
<thead>
<tr>
<th></th>
<th>Age (years)</th>
<th>Height (m)</th>
<th>Mass (kg)</th>
<th>BMI (kg/m$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SEM</td>
<td>21 ± 0.5</td>
<td>1.66 ± 0.02</td>
<td>60.3 ± 2.6</td>
<td>21.9 ± 0.9</td>
</tr>
<tr>
<td>Range</td>
<td>18 - 23</td>
<td>1.58 - 1.74</td>
<td>48.9 - 75.8</td>
<td>17.5 - 27.1</td>
</tr>
</tbody>
</table>

Table 3.4  Physical characteristics of the 11 subjects taking part in the pilot study. BMI (body mass index) = weight (kg)/height (m)$^2$.

VO$_2$max test: After an initial treadmill familiarisation VO$_2$max was determined directly for each subject using a modification of the protocol devised by Bruce (1972). This involved an incremental walking test which was divided into three minute stages. The speed of the treadmill remained constant throughout this test at either 1.56 m.s$^{-1}$ or 1.79 m.s$^{-1}$ (3.5 or 4.0 mph) depending on the subject's ability. The treadmill incline was set at 5% for the first three minutes, increased to 10% for the second three minutes and subsequently elevated by 2.5% at the end of each three minute period. Douglas bag samples of expired air were collected between minutes 1:45 and 2:45 of each stage of the test from which oxygen uptake and carbon dioxide production were measured.
Heart rate was monitored continuously throughout the test and the subject's PRE was measured at the end of each stage. The test was open ended and continued in the routine fashion described above until the subject signalled that she could only manage one more minute. At this point a final collection of expired air was taken and the test was terminated.

Submaximal incremental treadmill walking test: The purpose of this test was to determine the submaximal relationship between treadmill grade and oxygen uptake for each person. The test was 16 minutes long and was divided into four, four minute exercise periods. As with the VO$_2$max test the treadmill speed remained constant at either 1.56 m.s$^{-1}$ or 1.79 m.s$^{-1}$ during the test. The treadmill incline was set at an appropriate level for each individual to begin with and was subsequently elevated by 3% at the end of every stage. Expired air samples were collected for the last minute of each four minute stage of the test. The information gained from this test was used to calculate the treadmill grades required to elicit 60%, 70%, 80% and 90% of each subject's VO$_2$max, as described in Section 3.3.1 above. These grades were then employed in the third exercise test.

Grade-lactate treadmill test: This test was identical to that described in Section 3.3.2 except that the relative intensities used ranged from 60% to 90% of VO$_2$max rather than 50% to 80%. From the results of this test the oxygen uptake and relative exercise intensity attained at the reference blood lactate concentration of 4 mmol.l$^{-1}$ were ascertained as described above (Section 3.3.2).

Endurance walk: The purpose of this test was to make an assessment of endurance performance for each subject. An exercise intensity equivalent to 80% of VO$_2$max was chosen for the test in an attempt to ensure that fatigue was not primarily caused by substrate depletion but rather was related to other factors reflective of training status.

The test began with a five minute warm up period during which subjects walked at an incline required to elicit 60% of their VO$_2$max. At the end of this period the grade of the treadmill was increased so that each subject was exercising at 80% of their VO$_2$max and the test continued at this intensity until the point of volitional fatigue. Expired air was collected during the last minute of the warm up period and during the 80% walk between minutes four and
five, nine and ten and subsequently every ten minutes until a signal was given by the subject initiating a final one minute collection of expired air. Thumb prick samples of capillary blood were taken at rest and at the end of each expired air collection for the determination of blood lactate concentration. Heart rate was also monitored continuously as with the other exercise tests. A clock was placed in sight of the subjects during the walk so that they knew how long they had been walking for but the final times were not communicated between subjects in order to prevent their use as target times.

3.12.3 Results

A summary of the results for VO\textsubscript{2}max, VO\textsubscript{2} at 4 mmol.l\textsuperscript{-1}, %VO\textsubscript{2}max at 4 mmol.l\textsuperscript{-1} and endurance time is presented in Table 3.5. As can be seen from Table 3.5 the range in all of these variables is considerable and reflects the heterogeneous nature of the group.

<table>
<thead>
<tr>
<th>VO\textsubscript{2}max (ml.kg\textsuperscript{-1}min\textsuperscript{-1})</th>
<th>VO\textsubscript{2}:4 mmol.l\textsuperscript{-1} (ml.kg\textsuperscript{-1}min\textsuperscript{-1})</th>
<th>%VO\textsubscript{2}max at 4 mmol.l\textsuperscript{-1}</th>
<th>End. time (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SEM</td>
<td>41.9 ± 2.6</td>
<td>32.7 ± 3.1</td>
<td>76.7 ± 3.0</td>
</tr>
<tr>
<td>Range</td>
<td>26.8 - 54.4</td>
<td>17.6 - 47.6</td>
<td>62.2 - 89.2</td>
</tr>
</tbody>
</table>

Table 3.5 The mean ± SEM and range of values obtained for various physiological and performance variables assessed during the pilot study.

Figures 3.4, 3.5 and 3.6 demonstrate the relationships between endurance time and the other variables shown in Table 3.5 i.e. VO\textsubscript{2}max, VO\textsubscript{2} at 4 mmol.l\textsuperscript{-1} and the %VO\textsubscript{2}max at 4 mmol.l\textsuperscript{-1}. Due to the sample size involved in the study the Spearman rank order correlation coefficient (rho) was thought to be the most appropriate means of assessing the strength of association between these variables. All three relationships shown are significant although in each case there are two outliers away from the main group. These represent the two recreational runners who participated in the study. With the
omission of these subjects (i.e. n = 7) the relationships between VO$_2$max and endurance time and between the VO$_2$ at 4 mmol.l$^{-1}$ and endurance time remained significant (rho = 0.86 for both). However, the relationship between the %VO$_2$max at 4 mmol.l$^{-1}$ and endurance time became non-significant (rho = 0.71).

Figure 3.7 displays the relationship between the heart rate changes (10 minute values - 5 minute values) during the endurance walk and subsequent endurance time. Figure 3.8 shows the relationship between blood lactate concentration changes (10 minute values - 5 minute values) during the endurance walk and performance time. Only eight subjects are included in these Figures because one subject did not manage to walk for ten minutes. Both of these relationships were significant (rho = -0.78 and -0.93 respectively). With the removal of the two outliers the relationship between the heart rate changes and endurance time became non-significant (rho = -0.60) but the relationship between blood lactate concentration changes and endurance time remained significant (rho = -0.83).
Figure 3.4 The relationship between VO2max and time to exhaustion during treadmill walking for nine female subjects.
Figure 3.5 The relationship between the oxygen uptake attained at a blood lactate concentration of 4 mmol.l$^{-1}$ and time to exhaustion during treadmill walking for nine female subjects.
Figure 3.6  The relationship between the %VO$_2$max at a blood lactate concentration of 4 mmol.l$^{-1}$ and time to exhaustion during treadmill walking for nine female subjects.
Figure 3.7 The relationship between the heart rate change (10 minute values - 5 minute values) during a treadmill endurance walk and subsequent exhaustion time for eight female subjects.
Figure 3.8 The relationship between the blood lactate concentration change (10 minute values - 5 minute values) during a treadmill endurance walk and subsequent exhaustion time for eight female subjects.
3.12.4 Discussion

The fact that the relationship between VO\textsubscript{2max} and time to exhaustion during the endurance walk was so strong (Figure 3.4) was unexpected. Many studies have demonstrated a strong relationship between VO\textsubscript{2max} and endurance when performance is expressed in absolute terms i.e. running speed (Costill et al. 1973; Ramsbottom et al. 1987; Williams and Nute, 1983) but in this study endurance was assessed in relative terms i.e. time to exhaustion at 80% of VO\textsubscript{2max}. For this group of individuals then the possession of a high VO\textsubscript{2max} appeared to confer an improved ability to exercise at 80% of that VO\textsubscript{2max}. This conflicts with the findings of Mayes and co-workers (1987) which did not show a relationship between VO\textsubscript{2max} (I.min\textsuperscript{-1}) and time to exhaustion at 80% of VO\textsubscript{2max} during cycling exercise. However, as already mentioned the range of VO\textsubscript{2max} values in the present study was considerable (Table 3.5), the lowest value being 26.8 ml.kg\textsuperscript{-1}min\textsuperscript{-1} which would suggest a very sedentary person. It is likely then that the individuals in this study who possessed the higher maximum oxygen uptakes also exercised more frequently and thus were better trained than individuals with lower maximum oxygen uptakes.

The correlation between the 4 mmol.l\textsuperscript{-1} VO\textsubscript{2} and endurance time (Figure 3.5) was the same as that for VO\textsubscript{2max} and endurance time (rho = 0.93). This relationship may be explained by the fact that the 4 mmol.l\textsuperscript{-1} VO\textsubscript{2} is reflective of both VO\textsubscript{2max} and training status. In other words if two individuals attain the same relative intensity at a blood lactate concentration of 4 mmol.l\textsuperscript{-1}, the individual with the higher VO\textsubscript{2max} will be exercising at the higher oxygen uptake. Conversely, if two individuals possess similar VO\textsubscript{2max}'s then the one who is more highly trained will be able to exercise at a higher relative intensity at a blood lactate concentration of 4 mmol.l\textsuperscript{-1} and thus attain a higher oxygen uptake. This correlation between VO\textsubscript{2} at 4 mmol.l\textsuperscript{-1} and endurance time at 80% of VO\textsubscript{2max} is consistent with the findings from other studies which have demonstrated strong relationships between the running speed or work rate attained at a blood lactate concentration of 4 mmol.l\textsuperscript{-1} and performance time (Mayes et al. 1987; Sjodin and Jacobs, 1981; Williams and Nute, 1983).

For the group as a whole the 4 mmol.l\textsuperscript{-1} blood lactate concentration represented a relative exercise intensity of 77% (Table 3.5). This is lower than the value of 84% attained by the ten recreational runners examined by Williams and Nute (1983) probably due to differences in the two groups under
observation. Although the relationship between the %VO$_2$max attained at 4 mmol.l$^{-1}$ and endurance time was not as strong as that between either VO$_2$max or VO$_2$ at 4 mmol.l$^{-1}$ and endurance time it was still significant (rho = 0.85). Thus, individuals who could exercise at the highest relative intensities at a blood lactate concentration of 4 mmol.l$^{-1}$ were also best able to sustain walking at 80% of their VO$_2$max (Figure 3.6).

Figures 3.7 and 3.8 confirm the hypothesis that a rapid loss of cardiovascular and metabolic homeostasis is to some extent predictive of subsequent performance time. Both the changes in heart rate and blood lactate concentration between minutes five and ten of the endurance walk were significantly related to endurance time. This indicates that those individuals who experienced the greatest increase in either heart rate or blood lactate concentration between the fifth and tenth minutes of the endurance walk were likely to fatigue earliest and therefore have the shortest walking times. As mentioned in the results Section the relationship between heart rate changes and endurance time became non-significant when the values for the two recreational runners were excluded from the analysis. The relationship between blood lactate concentration changes and endurance time remained significant, although slightly weakened, when these two individuals were excluded. This suggests that changes in blood lactate concentration during the early stages of an endurance walk may be better predictors of subsequent performance than cardiovascular changes such as heart rate.

3.12.5 Conclusion

The results of this study show that the %VO$_2$max attained at a reference blood lactate concentration during submaximal walking can be used as an index of endurance fitness. There is no evidence from this study, however, to suggest that the %VO$_2$max attained at a reference blood lactate concentration is a better indicator of endurance fitness than VO$_2$max $per se$. This is probably due to the heterogeneous nature of the subject group and the likelihood that for these subjects VO$_2$max was reflective not only of genetic endowment but also habitual participation in physical activity.

On the basis of these findings it might be permissible to use both VO$_2$max and the %VO$_2$max attained at a reference blood lactate concentration as indices of endurance fitness in the middle-aged men participating in the brisk walking
study. However, because these men are required to be previously sedentary they should form a more homogeneous group in terms of habitual physical activity levels. Moreover, the training performed by the walkers in the brisk walking study may have little influence on VO$_2$max. If this is the case then the %VO$_2$max attained at a reference blood lactate concentration may provide a better index of endurance fitness (i.e. the ability to exercise at a given percentage of VO$_2$max) than VO$_2$max itself.

Finally, the results of this pilot study reveal that a rapid loss of cardiovascular and metabolic homeostasis during an endurance test are predictive of subsequent endurance performance. This suggests that changes in heart rate and blood lactate concentration in the endurance walk employed in the brisk walking study may be adopted as indices of endurance fitness.
4 PROCEDURES

4.1 Overview

The following Chapter describes the procedures used in the one year brisk walking study. This investigation involved the recruitment of a group of middle-aged men who were subsequently allocated into either a brisk walking group or a control group. The groups were then monitored during the following year in order to examine the effects of brisk walking on endurance fitness, lipoprotein metabolism and other selected risk factors for CHD. The study was a joint venture involving both the Department of Physical Education and Sports Sciences and the Department of Human Sciences at Loughborough University. Although each department had distinct and separate research interests many of the procedures described below were devised and accomplished jointly and the contribution of Miss Katherine Brooke-Wavell from the Department of Human Sciences is duly acknowledged.

4.2 Recruitment

All of the subjects participating in the study were volunteers and the majority came from Loughborough and the surrounding area although there were a few exceptions. Recruitment methods included the location of notices in doctor's and dentist's surgeries and other prominent places, advertisement in the local papers, participation in a health promotion day at Loughborough General Hospital and word of mouth. Although notices were posted around the University the response to these was limited and only a minority of subjects were University employees.

Once sufficient interest had been generated three meetings were held at the University which were open to anyone wishing to know more about the study. The methods and procedures to be employed were detailed at these meetings and written handouts were also produced explaining the study protocol (Appendix 1). If required, individual meetings were arranged giving the opportunity for further discussion and allowing personal queries and apprehensions to be addressed.
4.3 Screening

Following these meetings all men who were still interested in participating in the study were asked to make a preliminary visit to the laboratory for the purposes of screening. During this visit each subject's blood pressure was measured and a 2 ml sample of venous blood was collected for the determination of total cholesterol concentration. The session was concluded with a treadmill familiarisation in preparation for the exercise tests. Finally, all subjects were asked to visit the University Medical Officer for a routine medical examination.

4.4 Criteria for acceptance into the study

Following screening a decision was made as to whether to accept or reject each subject. Acceptance was based on the following criteria:

1. Male, aged between 42 and 59 years on entry into the study. These cut off points were chosen because the ageing process has little influence on the concentrations of lipids and lipoproteins within this range (Mann et al. 1988).

2. Neither currently involved in a programme of regular exercise nor employed in a strenuous job. This was assessed via a questionnaire.

3. Free from known cardiovascular disease as assessed by the University Medical Officer.

4. Not at high risk from CHD as determined by the major risk factors of cholesterol, blood pressure and smoking. Only non or occasional smokers were accepted on the study. High blood cholesterol was defined as >6.5 mmol.l\(^{-1}\) and high blood pressure was defined as >90 mm Hg for diastolic and >160 mm Hg for systolic. However, in a few subjects whose serum total cholesterol concentration was initially found to be below 6.5 mmol.l\(^{-1}\) subsequent values were higher (see Section 2.4 of Chapter Two on the variability in lipid and lipoprotein measurement). These subjects were not excluded from the study.
The above criteria provided a group of middle-aged men who were apparently healthy and currently sedentary.

4.5 Randomisation

A further criteria for entry into the study was the acceptance by the subjects of the principle of random allocation (in this case names were drawn from a hat) into either the walking or control group. This proved to be difficult for some individuals to accept because the motivation of many of the men for participating in the study was the desire to 'get fit'. However, randomisation was essential in order to prevent a self-selection effect. Therefore, to make this criterion more acceptable, a two to one ratio of walkers to controls was used, thus increasing each subject's likelihood of becoming a walker. Furthermore, the men who were allocated into the control group were promised the option of taking up an exercise programme with full monitoring once the first year of the study had been completed. Of course, an element of self-selection still existed since all of the subjects were volunteers and therefore were keen to participate in the study. Finally, it should be noted that the power of statistical tests will be reduced in this study because the experimental group is larger than the control group. This reduction should only be small, however, since Pocock (1983) estimates that a two to one ratio leads to a reduction of about 2.5% (i.e. from 95% to 92.5%) in statistical power.

4.6 Subjects

Around 100 men expressed a strong interest in participating in the study either over the telephone or by attending one of the open meetings arranged at the University. Seventy-nine of the men showed continued interest in the study after a full explanation of the procedures to be employed and the commitments necessary. A number of reasons were given for not wishing to enrol on the study. Some men were doubtful that they would have sufficient time to fulfil the obligations required of them while others were unwilling to accept the possibility of becoming a control subject. In addition two individuals were unwilling to undertake one or more of the procedures to be employed in the study i.e. the blood sampling and underwater weighing.
The 79 men who were still interested in participating in the study were now subjected to the screening process described above and six were rejected either due to ill health or because they exhibited an unfavourable risk factor profile for CHD. Another subject withdrew from the study when he learned of his inclusion in the control group. Seventy-two subjects actually started the study (48 walkers and 24 controls) and, of these, seven (six walkers and one control) dropped out at various stages during the following six months, leaving a group of 65 subjects who actually completed all of the procedures required.

Various factors contributed to the loss of subjects during the study. Two of the walkers had difficulty in attending the testing sessions because of work commitments and were unable to devote sufficient time to the exercise programme. Three subjects withdrew due to a combination of ill-health and motivational problems. One of the walkers suffered a recurrence of an old back injury and the control subject was forced to retire as a result of illness. A diagramatic representation of the numbers of men involved from initial recruitment through to the end of the study is given in Figure 4.1 and the physical characteristics of the 65 subjects who completed the study are shown in Table 4.1.

<table>
<thead>
<tr>
<th>100 men (approx.) expressed an interest in the study</th>
</tr>
</thead>
<tbody>
<tr>
<td>79 men attended screening for the study</td>
</tr>
<tr>
<td>73 men were accepted into the study</td>
</tr>
<tr>
<td>65 men completed the study</td>
</tr>
</tbody>
</table>

Figure 4.1 Summary of the number of men involved during each stage of the brisk walking study including recruitment, screening, acceptance and completion.
Physical characteristics of the 42 walkers and 23 controls who completed the brisk walking study. BMI (body mass index) = weight (kg)/height (m)$^2$. Values given are mean ± SEM and the range.

All of the subjects in Table 4.1 were Caucasian. The social class of these men was determined using the occupational classification system devised by the Joint Industry Committee for National Readership Surveys (Abrams, 1968). According to this classification system six subjects (9.2%) were graded upper middle-class, 34 subjects (52.3%) were graded middle-class, 20 subjects (30.8%) were graded lower middle-class and five subjects (7.7%) were graded skilled working class. None of the subjects were in the bottom two categories of this classification system i.e. semi-skilled/unskilled working class and those at the lowest levels of subsistence. Thus, the group were predominantly middle-class and lower middle-class by occupation.

4.7 Study protocol

Approval for the procedures described below was granted by the University ethical committee.

4.7.1 Structure of the study

The study protocol involved testing the subjects at base line and after three, six and 12 months of participation in the study. Since the group was too large to deal with at any one time it was split into three smaller cohorts each containing 16 walkers and eight controls. The inclusion of both walkers and controls in each cohort ensured that no systematic differences would occur
between groups because of the cohort approach. It was anticipated that it would take at least one month to test each cohort thus testing began on cohort one with cohort two starting a month later and cohort three starting two months later. Once the tests on cohort three had been completed the cycle began again with cohort one followed by cohort two etc. A summary of this protocol is shown below (Table 4.2).

<table>
<thead>
<tr>
<th>Year</th>
<th>Month</th>
<th>Cohort</th>
<th>Testing period</th>
</tr>
</thead>
<tbody>
<tr>
<td>1989</td>
<td>April</td>
<td>1</td>
<td>Base line</td>
</tr>
<tr>
<td></td>
<td>May</td>
<td>2</td>
<td>Base line</td>
</tr>
<tr>
<td></td>
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<td>3</td>
<td>Base line</td>
</tr>
<tr>
<td></td>
<td>July</td>
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<td>6 months</td>
</tr>
<tr>
<td></td>
<td>November</td>
<td>2</td>
<td>6 months</td>
</tr>
<tr>
<td></td>
<td>December</td>
<td>3</td>
<td>6 months</td>
</tr>
<tr>
<td>1990</td>
<td>January</td>
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<td></td>
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<td>March</td>
<td>-</td>
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<tr>
<td></td>
<td>April</td>
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<td>12 months</td>
</tr>
<tr>
<td></td>
<td>May</td>
<td>2</td>
<td>12 months</td>
</tr>
<tr>
<td></td>
<td>June</td>
<td>3</td>
<td>12 months</td>
</tr>
</tbody>
</table>

Table 4.2 Timescale over which the brisk walking study was conducted.

4.7.2 Test battery

The battery of tests completed by the subjects varied according to the testing interval (i.e. base line, three, six or 12 months). The base-line tests were the most extensive and included the following:
Exercise tests: Submaximal-incremental treadmill walking test
Grade-lactate treadmill walking test
Treadmill endurance walk
Track walk

Blood samples for the determination of: Total cholesterol
HDL-C
LDL-C
VLDL-C
Triglycerides
Apoproteins A-I and B
Lp(a)
Fibrinogen
Haemoglobin
Haematocrit

Resting arterial blood pressure: Systolic blood pressure
Diastolic blood pressure

Lung function measures: FVC
FEV₁
FVC/FEV₁ (%)

Seven day weighed food inventory

Anthropometry and body composition: Height
Body mass
Skinfold thicknesses
Circumferences
Body density (hydrostatic weighing)
Most of these tests were repeated after three, six and 12 months with the following exceptions. The submaximal-incremental treadmill test was discontinued having been used simply to aid in the development of the grade-lactate treadmill test and the endurance walk. Lipoprotein(a) was not measured at three and six months due to expense. The subjects were not required to complete a seven day weighed food inventory at the three month stage. Finally, hydrostatic weighing (used for the determination of body density) was not performed at three and six months due to the time consuming nature of this measurement and the limited number of technical staff available. This thesis is primarily concerned with the data regarding the exercise testing, blood biochemistry, arterial blood pressure and lung function measures. The nutritional data and the data regarding anthropometry and body composition are discussed in detail by Brooke-Wavell (1992).

4.8 Brisk walking

4.8.1 The brisk walking programme

After the base-line tests had been completed the control subjects were asked to maintain their habitual lifestyle while the subjects in the exercise group began the walking programme. Both groups were asked not to make any planned dietary changes during the study. The exercise regimen prescribed during the study was based on sound training principles such as those provided by the American College of Sports Medicine (ACSM) (1991). The walkers were given two targets to aim for during the study. Over the first three months they were asked to gradually build up their training until they were managing to average between 20 and 25 minutes of brisk walking per day. They were then required to increase this amount to between 40 and 45 minutes of brisk walking per day over the next three months and to maintain this level thereafter until the end of the study. In order to achieve these targets it was not necessary for the subjects to walk every day, they could do more walking on some days and less on others if they wished. They were also allowed rest days provided they made the time up on other days. However, there were certain provisos and these included the following:
1. A minimum of three walks per week were required during the first month of the training programme. This was increased to four and five walks per week for the second and third months respectively. Although there were no further stipulations in this respect most men found that they needed to walk at least six days per week to meet the six month walking target.

2. The minimum length of any one walk was set at 20 minutes, anything less than this did not constitute part of the training programme.

3. The walking had to be brisk. This was defined for the men as a pace which was faster than they normally walked but not 'flat out' and which increased their breathing beyond the normal rate.

4. The walking had to be additional to any walking which was usually performed during the day.

The subjects were also cautioned as to the dangers of overtraining. Thus, they were warned not to progress their training mileage or intensity too suddenly and to ease off in cases of illness or injury and forget about the training targets until they had made a full recovery. They were also advised on footwear, walking surfaces and any other aspect of the training programme which they wished to discuss.

According to Haskell (1986) an exercise regimen requiring an increase in energy expenditure of 5 MJ (1200 kcal) per week will often be sufficient to stimulate an increase in HDL-C concentration in previously sedentary healthy persons. Previous research has shown that the energy cost of horizontal walking at 4.0 mph is around 6.4 kcal per minute for men weighing 82 kg (McArdle et al. 1981). Assuming a similar walking speed but adjusting for body mass (i.e. 79.1 kg for the walking group) the estimated energy expenditure during walking, for the men in the present study, would be approximately 6.2 kcal per minute or 93 kcal per mile. Moreover, if the training programme outlined above were rigidly adhered to the subjects in this study would be walking an average of 10.5 miles per week after three months and 21.0 miles per week after six months. This would result in a weekly energy expenditure of 4.1 MJ (977 kcal) and 8.2 MJ (1953 kcal) at three and six months respectively according to the above calculations. This exercise regimen might therefore, on
the basis of the existing literature, be expected to influence lipoprotein metabolism.

4.8.2 Monitoring of the brisk walking programme

There were no structured training sessions during the study. The men incorporated the walking into their daily routines however they saw fit. Variations included walking before or after work, walking during the lunch hour or walking to and from work. Some men performed longer walks on the weekends in order to achieve the targets set, others simply walked the same distance every day. Training progress was monitored by means of self-report. Walkers were given diaries (an example is shown in Appendix 15) which they completed and sent to the University at the end of every month. Each diary sheet covered a two week period and subjects were asked to try and maintain the walking averages stipulated during each fortnight. The men were continually reminded of the importance of accurate recording. Thus, although the walkers were encouraged to achieve the targets set on the walking programme these were not stressed to the extent of encouraging false reporting.

4.8.3 Adherence to the brisk walking programme

Actual adherence to the walking programme is shown in Figures 4.2 and 4.3. Figure 4.2 gives the average minutes of brisk walking performed each day during the study. Data are divided into four week periods and for each of these periods the 25th, 50th and 75th percentiles are given together with the minimum and maximum values. Figure 4.3 displays the average minutes of brisk walking performed each day by each subject over the study year. For the group as a whole the average minutes of brisk walking performed each day over the training year was 27.9 ± 9.2 (mean ± SD) (range 10.9 - 46.6 mins per day). Although this fell short of the target set it still amounted to some 14.1 ± 4.7 miles of brisk walking per week (calculated using an average brisk walking pace of 4.4 mph assessed via the track walks).
Figure 4.2 The average minutes of brisk walking per day completed each four weeks by the 42 subjects in the brisk walking group.
Figure 4.3 The average minutes of brisk walking per day for each subject during the one year training period.
5 CARDIOVASCULAR AND METABOLIC ADAPTATIONS TO BRISK WALKING

5.1 Introduction

This Chapter examines the extent to which the brisk walking programme elicited changes in the cardiovascular and metabolic responses of the walkers to submaximal exercise. Improvements in either or both of these factors would indicate an enhanced potential for endurance exercise in the walking group.

5.2 Methods

The data presented in this Chapter relates to the exercise tests described in Chapter Three, namely the grade-lactate treadmill test (3.3.2), the endurance walk (3.3.3) and the track walk (3.3.4). All three of these tests were performed at base line and repeated after three, six and 12 months as described in Chapter Four (4.7).

Each exercise test had a specific function. The grade-lactate treadmill test allowed for an assessment of the physiological and metabolic responses to steady state exercise at four different intensities. These intensities were initially set at 50%, 60%, 70%, and 80% of predicted VO$_2$max by manipulating the treadmill incline and speed. However, although the inclines and speeds used for each subject remained constant throughout the study, the relative intensities elicited would have been altered if there were any changes in VO$_2$max.

The endurance walk was conducted to facilitate an examination of the subjects' physiological and metabolic responses to steady state exercise (70% of predicted VO$_2$max at base line) over a 20 minute period. This provided a means of determining if the brisk walking programme influenced the subjects' ability to maintain steady state responses i.e. whether or not there was any reduction in the homeostatic disturbance caused by exercise. Finally, the track walk was conducted in an attempt to assess brisk walking speed in the walking group and whether or not this speed changed during the study. The track walk also provided a simple index (heart rate) of the physiological response to brisk walking.
The responses of the walkers and controls were compared primarily using two-way analysis of variance for repeated measures. For the PRE data the Mann Whitney U test was used to detect differences between walkers and controls while the Wilcoxon matched pairs test was employed to examine changes over time within each group. This was due to the fact that the PRE data was ordinal in nature. Relationships between variables were examined using the Pearson Product Moment correlation coefficient.

For data displayed in Tables or Figures the number of subjects in each group is 42 and 23 for walkers and controls respectively unless otherwise stated. Throughout this Chapter the following abbreviations are used in Tables: 'W': walkers; 'C': controls; 'BL': base line and 'M': months.

5.3 Results

Oxygen uptake values (ml.kg\(^{-1}\)min\(^{-1}\)) during the grade-lactate treadmill test are shown in Table 5.1. For the walkers, 12 month values for oxygen uptake were on average 3.0 ml.kg\(^{-1}\)min\(^{-1}\) (13.2%) lower than base-line values during each stage of the grade-lactate test. In the control group oxygen uptake was also reduced but the average reduction was not as great at 2.5 ml.kg\(^{-1}\)min\(^{-1}\) (11.5%) and there were significant interactions between walkers and controls at stages two and three of the grade-lactate test. Reductions in oxygen consumption were also seen during the study when values were expressed in l.min\(^{-1}\) but body weight remained relatively stable throughout the study (Table 5.2).
<table>
<thead>
<tr>
<th>Stage</th>
<th>W BL</th>
<th>W 3M</th>
<th>W 6M</th>
<th>W 12M</th>
<th>C BL</th>
<th>C 3M</th>
<th>C 6M</th>
<th>C 12M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17.3 ± 0.4</td>
<td>16.4 ± 0.5</td>
<td>15.3 ± 0.4</td>
<td>14.6 ± 0.4</td>
<td>16.9 ± 0.7</td>
<td>16.8 ± 0.8</td>
<td>15.7 ± 0.7</td>
<td>14.7 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>20.5 ± 0.5</td>
<td>19.7 ± 0.5</td>
<td>18.9 ± 0.5</td>
<td>17.8 ± 0.5</td>
<td>19.6 ± 0.8</td>
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<td>19.0 ± 0.8</td>
<td>17.1 ± 0.8</td>
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<tr>
<td></td>
<td>24.6 ± 0.5</td>
<td>23.6 ± 0.6</td>
<td>22.8 ± 0.6</td>
<td>21.6 ± 0.5</td>
<td>23.5 ± 1.0</td>
<td>24.1 ± 1.1</td>
<td>23.1 ± 1.0</td>
<td>21.0 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>29.1 ± 0.6</td>
<td>28.3 ± 0.6</td>
<td>27.4 ± 0.6</td>
<td>25.7 ± 0.6</td>
<td>27.8 ± 1.1</td>
<td>28.0 ± 1.2</td>
<td>27.2 ± 1.1</td>
<td>25.2 ± 1.1</td>
</tr>
</tbody>
</table>

Table 5.1 Oxygen uptake (ml.kg⁻¹min⁻¹) for walkers and controls during the last minute of each stage of an incremental treadmill walking test performed at baseline and after three, six and 12 months of the brisk walking study (mean ± SEM). Interaction significant at stages 2 and 3.
Table 5.2  Body weight (kg) during the brisk walking study for walkers and controls (mean ± SEM). Interaction not significant.

<table>
<thead>
<tr>
<th></th>
<th>Base line</th>
<th>3 months</th>
<th>6 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>W</td>
<td>79.1 ± 1.5</td>
<td>78.4 ± 1.5</td>
<td>79.2 ± 1.5</td>
<td>78.9 ± 1.5</td>
</tr>
<tr>
<td>C</td>
<td>78.2 ± 2.5</td>
<td>77.6 ± 2.5</td>
<td>78.8 ± 2.5</td>
<td>78.9 ± 2.7</td>
</tr>
</tbody>
</table>

Table 5.2  Body weight (kg) during the brisk walking study for walkers and controls (mean ± SEM). Interaction not significant.
The heart rate attained during treadmill exercise was reduced in both the walking and control groups as the study progressed. However, as indicated in Table 5.3 the reduction in heart rate was greater in the walking group than in the control group. Thus, the average reduction in heart rate after 12 months was 9 b.min⁻¹ (6.7%) for the walkers and 5 b.min⁻¹ (3.8%) for the controls at each stage of the grade-lactate treadmill test and there was a significant interaction at all stages of the test. Individual heart rate changes during the grade-lactate test after three, six and 12 months were highly variable ranging from -27 to +19 b.min⁻¹ and from -30 to +27 b.min⁻¹ in the walking and control groups respectively. Heart rate changes in the endurance walk followed a similar pattern to those in the grade-lactate test (Table 5.4) and a significant interaction was obtained for minutes 14 to 15.
<table>
<thead>
<tr>
<th>Stage:</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>W BL</td>
<td>111 ± 1</td>
<td>122 ± 1</td>
<td>137 ± 1</td>
<td>153 ± 1</td>
</tr>
<tr>
<td>W 3M</td>
<td>106 ± 1</td>
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</tr>
<tr>
<td>W 6M</td>
<td>104 ± 1</td>
<td>116 ± 1</td>
<td>129 ± 1</td>
<td>145 ± 1</td>
</tr>
<tr>
<td>W 12M</td>
<td>103 ± 1</td>
<td>114 ± 1</td>
<td>128 ± 1</td>
<td>143 ± 1</td>
</tr>
<tr>
<td>C BL</td>
<td>110 ± 2</td>
<td>122 ± 2</td>
<td>136 ± 2</td>
<td>151 ± 2</td>
</tr>
<tr>
<td>C 3M</td>
<td>109 ± 1</td>
<td>121 ± 1</td>
<td>135 ± 2</td>
<td>150 ± 2</td>
</tr>
<tr>
<td>C 6M</td>
<td>108 ± 2</td>
<td>121 ± 2</td>
<td>135 ± 2</td>
<td>152 ± 2</td>
</tr>
<tr>
<td>C 12M</td>
<td>104 ± 1</td>
<td>117 ± 1</td>
<td>132 ± 2</td>
<td>147 ± 2</td>
</tr>
</tbody>
</table>

Table 5.3 Heart rate (b.min⁻¹) for walkers and controls during the last minute of each stage of an incremental treadmill walking test performed at base line and after three, six and 12 months of the brisk walking study (mean ± SEM). Interaction significant at all stages.
<table>
<thead>
<tr>
<th>Minutes:</th>
<th>4-5</th>
<th>9-10</th>
<th>14-15</th>
<th>19-20</th>
</tr>
</thead>
<tbody>
<tr>
<td>W BL</td>
<td>134 ± 1</td>
<td>140 ± 1</td>
<td>143 ± 1</td>
<td>145 ± 1</td>
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<tr>
<td>W 3M</td>
<td>130 ± 1</td>
<td>135 ± 2</td>
<td>138 ± 2</td>
<td>141 ± 2</td>
</tr>
<tr>
<td>W 6M</td>
<td>129 ± 1</td>
<td>135 ± 1</td>
<td>138 ± 1</td>
<td>141 ± 1</td>
</tr>
<tr>
<td>W 12M</td>
<td>125 ± 2</td>
<td>130 ± 2</td>
<td>133 ± 2</td>
<td>135 ± 2</td>
</tr>
<tr>
<td>C BL</td>
<td>134 ± 2</td>
<td>140 ± 2</td>
<td>144 ± 2</td>
<td>147 ± 2</td>
</tr>
<tr>
<td>C 3M</td>
<td>132 ± 2</td>
<td>139 ± 2</td>
<td>143 ± 2</td>
<td>146 ± 3</td>
</tr>
<tr>
<td>C 6M</td>
<td>132 ± 2</td>
<td>139 ± 2</td>
<td>143 ± 2</td>
<td>146 ± 2</td>
</tr>
<tr>
<td>C 12M</td>
<td>130 ± 2</td>
<td>136 ± 3</td>
<td>141 ± 2</td>
<td>144 ± 2</td>
</tr>
</tbody>
</table>

Table 5.4 Heart rate (b.min⁻¹) for walkers and controls during 20 minutes of treadmill walking (70% of VO₂max) at base line and after three, six and 12 months of the brisk walking study (mean ± SEM). Interaction significant for minutes 14-15.
Although the reductions in heart rate and oxygen uptake were not much greater in the walkers than they were in the controls, when these two parameters were examined together the differences between the two groups became more apparent. This is demonstrated in Figure 5.1 which shows the heart rate/oxygen uptake relationship from the grade-lactate treadmill test for walkers and controls. In the case of the walkers this relationship remained largely unchanged throughout the study whereas the control group experienced an increased heart rate for a given oxygen uptake after 12 months as compared to base line.
Figure 5.1 The oxygen uptake/heart rate relationship during a four staged treadmill walking test at base line and after 12 months of the brisk walking study for walkers and controls (mean ± SEM).
Blood lactate concentration during the grade-lactate test is presented in Table 5.5 and Figure 5.2. As with the heart rate responses there were reductions in both the walkers and controls but changes were greater in the walkers and there were significant interactions at stages two, three and four of the test. In both groups the greatest reductions were seen after six months. In the walkers these reductions averaged 1.1 mmol.l⁻¹ (36.8%) at each stage of the grade-lactate test while control group reductions averaged 0.3 mmol.l⁻¹ (11.6%). Although 12 month blood lactate concentrations were slightly higher than six month values they were still around 30% lower in the walkers than the corresponding base-line values. The 12 month blood lactate values for the controls were around 13% lower than the corresponding base-line values. For both the walkers and controls the changes in blood lactate concentration during the endurance walk were of a similar magnitude to those observed in the grade-lactate test (Table 5.6) and significant interactions were obtained at minutes four to five, nine to ten and 19 to 20.
<table>
<thead>
<tr>
<th>Stage:</th>
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<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>W BL</td>
<td>1.8 ± 0.1</td>
<td>2.2 ± 0.2</td>
<td>3.1 ± 0.2</td>
<td>4.9 ± 0.3</td>
</tr>
<tr>
<td>W 3M</td>
<td>1.4 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>2.1 ± 0.2</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>W 6M</td>
<td>1.2 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.9 ± 0.2</td>
<td>3.0 ± 0.2</td>
</tr>
<tr>
<td>W 12M</td>
<td>1.5 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td>C BL</td>
<td>1.5 ± 0.1</td>
<td>2.0 ± 0.2</td>
<td>2.9 ± 0.3</td>
<td>4.6 ± 0.5</td>
</tr>
<tr>
<td>C 3M</td>
<td>1.6 ± 0.1</td>
<td>2.0 ± 0.2</td>
<td>2.8 ± 0.3</td>
<td>4.3 ± 0.5</td>
</tr>
<tr>
<td>C 6M</td>
<td>1.4 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>2.5 ± 0.2</td>
<td>4.1 ± 0.3</td>
</tr>
<tr>
<td>C 12M</td>
<td>1.4 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>2.6 ± 0.2</td>
<td>4.0 ± 0.4</td>
</tr>
</tbody>
</table>

Table 5.5 Blood lactate concentration (mmol·l⁻¹) for walkers and controls at the end of each stage of an incremental treadmill walking test performed at baseline and after three, six and 12 months of the brisk walking study (mean ± SEM). Interaction significant at stages 2, 3 and 4.
Figure 5.2 Blood lactate concentration at the end of each stage of an incremental treadmill walking test at base line and after 12 months of the brisk walking study for walkers and controls (mean ± SEM).
Table 5.6 Blood lactate concentration (mmol.l\(^{-1}\)) for walkers and controls during a 20 minute treadmill walk (70% of VO\(_{2}\)max) at base line and after three, six and 12 months of the brisk walking study (mean ± SEM). Interaction significant for minutes 4-5, 9-10 and 19-20.
Table 5.7 displays the range of change in blood lactate concentration at each stage of the grade-lactate test. The values shown in this Table were obtained by subtracting base-line values from values subsequently obtained at three, six and 12 months. From this Table it can be seen that the range of change is quite large in the control group after three, six and 12 months with both increases and decreases in blood lactate concentration occurring at all stages of the test. A similar pattern emerged in the walking group for the first two stages of the test. During stages three and four of the test, however, the direction of the response was much more uniform in the walking group. As shown in Table 5.7 six month changes in blood lactate concentration for the walkers ranged from -4.6 to +0.8 and from -6.9 to +0.3 mmol.l⁻¹ for stages three and four respectively. After 12 months equivalent values ranged from -3.8 to +0.4 and -5.3 to +0.6 mmol.l⁻¹. Thus, practically all of the walkers experienced reductions in blood lactate concentration during the last two stages of the grade-lactate treadmill test at six and 12 months and in one subject a decrease of almost 7 mmol.l⁻¹ was observed.
Table 5.7 The range of change in blood lactate concentration (mmol.L⁻¹) at each stage of an incremental treadmill walking test performed after three, six and 12 months of the brisk waking study for walkers and controls. Values were obtained by subtracting base-line concentrations from concentrations at three, six and 12 months.

<table>
<thead>
<tr>
<th>Stage:</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>W 3M - BL</td>
<td>-3.3/ +4.0</td>
<td>-2.5/ +3.4</td>
<td>-3.6/ +1.3</td>
<td>-4.9/ +3.1</td>
</tr>
<tr>
<td>W 6M - BL</td>
<td>-3.5/ +2.1</td>
<td>-3.9/ +1.7</td>
<td>-4.6/ +0.8</td>
<td>-6.9/ +0.3</td>
</tr>
<tr>
<td>W 12M - BL</td>
<td>-3.1/ +2.8</td>
<td>-2.9/ +1.6</td>
<td>-3.8/ +0.4</td>
<td>-5.3/ +0.6</td>
</tr>
<tr>
<td>C 3M - BL</td>
<td>-1.2/ +1.3</td>
<td>-1.1/ +1.8</td>
<td>-1.6/ +1.7</td>
<td>-2.7/ +2.3</td>
</tr>
<tr>
<td>C 6M - BL</td>
<td>-1.1/ +1.3</td>
<td>-1.5/ +1.5</td>
<td>-2.8/ +1.6</td>
<td>-4.2/ +2.7</td>
</tr>
<tr>
<td>C 12M - BL</td>
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<td>-1.4/ +0.5</td>
<td>-1.4/ +1.0</td>
<td>-2.8/ +1.4</td>
</tr>
</tbody>
</table>
Figure 5.3 shows that the relationship between blood lactate concentration and oxygen uptake during the grade-lactate treadmill test has changed in opposite directions for walkers and controls. In the case of the walkers blood lactate concentration decreased for a given oxygen uptake after 12 months as compared to base line whereas in the control group blood lactate concentration was higher for a given oxygen uptake after 12 months. As a consequence of this changed relationship the oxygen uptake attained at a blood lactate concentration of 2 mmol.l⁻¹ increased significantly during the study for the walkers but not for the controls (Table 5.8). The percentage of predicted VO₂max attained at a blood lactate concentration of 2 mmol.l⁻¹ also tended to increase for the walkers but their response did not differ significantly from that of the controls (Table 5.8).
Figure 5.3 The oxygen uptake/blood lactate relationship during a four staged treadmill walking test at base line and after 12 months of the brisk walking study for walkers and controls (mean ± SEM).
Predictions of VO$_2$max (ml.kg$^{-1}$min$^{-1}$) from the heart rate/oxygen uptake data collected during the grade-lactate treadmill test are shown in Table 5.9. At base line predicted VO$_2$max values ranged from 25.8 ml.kg$^{-1}$min$^{-1}$ to 46.8 ml.kg$^{-1}$min$^{-1}$ in the walking group and from 25.9 ml.kg$^{-1}$min$^{-1}$ to 45.4 ml.kg$^{-1}$min$^{-1}$ in the control group. Values for predicted VO$_2$max remained relatively stable in the walking group throughout the study but there was an 8.5% decrease in predicted VO$_2$max in the control group by the end of the study. The interaction effect was significant. Within the walking group individual changes in VO$_2$max during the study ranged from -4.7 to +10.3 ml.kg$^{-1}$min$^{-1}$. 
<table>
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<tr>
<th></th>
<th>Base line</th>
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<th>6 months</th>
<th>12 months</th>
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<td>36.8 ± 0.8</td>
<td>37.0 ± 0.9</td>
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<td>C</td>
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<td>35.4 ± 1.1</td>
<td>33.6 ± 1.1</td>
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Table 5.9 Predicted VO$_2$max values (ml.kg$^{-1}$min$^{-1}$) for walkers and controls during the brisk walking study (mean ± SEM). Interaction significant.
Figure 5.4 displays the ventilatory equivalent for oxygen values (VE.\(\text{VO}_2\)\(^{-1}\)) obtained during the grade-lactate treadmill test at base line and after 12 months. There was an increase in VE.\(\text{VO}_2\)\(^{-1}\) values for both walkers and controls during the study but the increase was greater in the controls than it was in the walkers and there were significant interactions at stages two, three, and four of the grade-lactate test. The increased VE.\(\text{VO}_2\)\(^{-1}\) values were related to the fact that oxygen uptake (l.min\(^{-1}\)) decreased during treadmill exercise in both the walking and control groups (Figure 5.5) without equivalent reductions in the rate of ventilation (Figure 5.6).

The R values obtained during the grade-lactate test at base line and after 12 months are shown in Figure 5.7. For the walkers, R values were decreased slightly after 12 months whereas there was a clear elevation in the R values of the control group after 12 months and significant interactions were obtained at stages two, three and four of the test.

Analysis via the Mann Whitney U test did not reveal any differences in PRE values between walkers and controls at any point during the study. Within each group, however, there were significant changes from base-line values, assessed using the Wilcoxon matched pairs test. The PRE values obtained from the grade-lactate treadmill test for both walkers and controls are shown in Figure 5.8.

Data from the track walk is presented in Table 5.10. This includes the average time taken to complete the walk, the average walking speed and the average heart rate and percentage of maximum heart rate attained during the walk over the course of the year. There were no significant interactions between the walkers and controls for any of these variables.
Figure 5.4 Ventilatory equivalent for oxygen values (VE/VO2) during an incremental treadmill walking test at base line and after 12 months of the brisk walking study for walkers and controls (mean ± SEM).
Figure 5.5 Oxygen uptake (VO2) during the last minute of each stage of an incremental treadmill walking test at base line and after 12 months of the brisk walking study for walkers and controls (mean ± SEM).
Figure 5.6 Ventilation (VE) during the last minute of each stage of an incremental treadmill walking test at base line and after 12 months of the brisk walking study for walkers and controls (mean ± SEM).
Figure 5.7  Respiratory exchange ratio (R) values during each stage of an incremental treadmill walking test at base line and after 12 months of the brisk walking study for walkers and controls (mean ± SEM).
Figure 5.8 Mean PRE values during an incremental treadmill walking test at base line and after 12 months of the brisk walking study for walkers and controls. * indicates significance from base line (Wilcoxon test).
<table>
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<tr>
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</tr>
</thead>
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<tr>
<td>Walk time (minutes)</td>
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</tr>
<tr>
<td>HR (b.min⁻¹)</td>
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<td>119 ± 2</td>
<td>121 ± 2</td>
<td>119 ± 3</td>
</tr>
<tr>
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<td>65.2 ± 1.9</td>
<td>69.0 ± 1.7</td>
<td>67.9 ± 1.9</td>
</tr>
</tbody>
</table>

Table 5.10 Data from the 1600m track walk for walkers and controls. Variables shown are walk time, walking speed and the average heart rate (HR) and percentage of maximum heart rate (%HRmax) at base line, three, six and 12 months (mean ± SEM). No significant interactions.
5.4 Discussion

As discussed earlier (Section 4.8.3) the walkers had varying degrees of success in adhering to the training programme. This is demonstrated by the large range in the minutes of brisk walking completed during the training year which extended from 3,966 to 16,959 (or an average of 11 to 47 minutes per day). However, the targets set for the brisk walking programme were very demanding and perhaps a little unrealistic bearing in mind the other commitments which many of the men had (i.e. work/family etc.). Nevertheless, the amount of training completed by the group was impressive and the average subject completed 10,154 minutes of brisk walking during the study which equated to 27.9 minutes (or approximately two miles) of brisk walking every day for one year. This is considerably more than the amount of training achieved in most other short or long term training studies.

The average brisk walking speed of the walking group spanned from 1.90 m.s\(^{-1}\) (4.3 mph) at base line to 1.98 m.s\(^{-1}\) (4.4 mph) after 12 months (Table 5.10). These speeds are within the range proposed as being necessary for adult men to achieve cardiorespiratory benefit (i.e. 1.78 m.s\(^{-1}\) to 2.00 m.s\(^{-1}\) or 4.0 mph to 4.5 mph) (Porcari et al. 1989). The average training intensity of the walking group remained unchanged throughout the study at around 68% of maximum heart rate. This is very close to the criterion training intensity used by Porcari and co-workers (1987) in their walking studies (i.e. 70% of HRmax) and is within the range of intensity suggested by the ACSM (1991) as being optimal in terms of improving cardiorespiratory endurance (i.e. 40% to 85% of VO\(_2\)max or 55% to 90% of HRmax). However, the range of individual training intensities attained by the walkers during the track tests was large extending from 53% to 88% of HRmax. Thus, most though not all of the walkers were conforming to the ACSM guidelines.

Within the training group walking speed correlated significantly with predicted VO\(_2\)max at base line (\(r = 0.41\)), 3 months (\(r = 0.47\)), 6 months (\(r = 0.45\)) and 12 months (\(r = 0.49\)). No significant correlations were obtained between age and brisk walking speed. These results are consistent with those of Hudson (1991) who also found a significant correlation between brisk walking speed and predicted VO\(_2\)max but not age in her female walkers.
The reductions in oxygen consumption which occurred in both groups of subjects indicate that energy expenditure decreased at a given treadmill incline as the study progressed. This finding is important and partly explains the changes in some of the other variables measured during the treadmill tests.

It is likely that the reduction in oxygen consumption during submaximal treadmill walking was primarily due to treadmill habituation. Although all of the study participants were familiarised on the treadmill before the study commenced the familiarisation was not extensive and it appears that the subjects continued to improve their treadmill walking economy (mechanical efficiency) throughout the study. As can be seen from Table 5.1 the reduction in submaximal oxygen uptake is gradual and progressive during the study supporting this last point. Improvements in mechanical efficiency would result from changes in walking gait such that vertical displacement is reduced to a minimum and only the necessary musculature is recruited in the walking action. These improvements would be achieved via adjustments in stride length and frequency until both were at an optimum (Lamb, 1984; McArdle et al. 1981).

As mentioned above the walkers experienced a greater reduction in submaximal oxygen consumption during the grade-lactate treadmill test than the controls. This is advantageous since it means that the relative exercise intensity is reduced and therefore the stress of exercise decreased. The difference between the two groups was only small (an average of 0.5 ml.kg⁻¹min⁻¹) but is nevertheless important and may be attributed to the brisk walking programme since the walkers did no more treadmill walking than the controls. This conclusion is supported by the results of some running studies which have shown that trained runners are more efficient (consume less oxygen at a given submaximal running speed) than untrained subjects (Bransford and Howley, 1977; Margaria et al. 1963). Moreover, running economy has been shown to improve with training (Conley et al. 1984) and the number of years engaged in training was significantly related to running efficiency in a group of nine male distance runners examined by Mayhew (1977).
There is little information regarding changes in submaximal oxygen consumption over a period of walking training although some does exist. Santiago and colleagues (1987), for example, found significant reductions in submaximal oxygen consumption in a group of previously sedentary women after 20 weeks of walking training. In contrast to this, however, Seals and co-workers (1984a) reported that oxygen uptake at the same absolute work rate was unaffected by six months of walking training and Hudson (1991) found no differences between the submaximal oxygen consumption of female walkers and controls during a one year training study.

Within the walking group the reductions in heart rate (Table 5.3) during each stage of the grade-lactate test, which averaged 6.7% after 12 months, suggest some degree of cardiovascular adaptation. These findings are consistent with those of other walking studies which have shown decreases in heart rate of between 4.2% and 11.0% during submaximal exercise after various periods of training (Hudson, 1991; Jette et al. 1988; Pollock et al. 1971; Santiago et al. 1987; Seals et al. 1984a). However, the changes found in the present study are relatively small bearing in mind the fact that the walkers' submaximal oxygen uptake was decreased after training by an average of 13.2% during the grade-lactate test. Moreover, if the percentage change in heart rate for the control group is deducted from the percentage change in heart rate for the walking group, the net reduction in heart rate for the walkers is 2.9% (4 b.min⁻¹).

It will be recalled from Section 5.2 that the purpose of the endurance walk was to assess whether or not there were any changes in the walkers' ability to maintain steady state responses during exercise. As can be seen from Table 5.4 heart rate increased progressively during the endurance walk both at base line and after 12 months and although heart rate is lower throughout the test after 12 months as compared to base line the magnitude of the increase from five minutes to 20 minutes is still the same i.e. around 8%. Thus, although the brisk walking programme did lead to reductions in heart rate during the endurance walk, the actual increase in heart rate over time remained unchanged.

Within the walking group changes in blood lactate concentration during the study were profound and thus provide evidence of peripheral (skeletal) adaptations to exercise training. Reductions were particularly great during the
last two stages of the grade-lactate treadmill test. After 12 months for example, the average reductions in blood lactate concentration for the walkers at the end of stages three and four were 1.1 mmol.L\(^{-1}\) (35.5%) and 1.8 mmol.L\(^{-1}\) (36.7%) respectively (Table 5.5). Even if the reductions in the control group are deducted from these values the net changes in the walkers still amount to 0.8 mmol.L\(^{-1}\) (25.2%) and 1.2 mmol.L\(^{-1}\) (23.7%) for stages three and four respectively.

The findings from this investigation are consistent with those from former studies examining running (Hurley et al. 1984) cycling (Mayes et al. 1987) and walking (Seals et al. 1984b; Hudson, 1991), all of which have shown that blood lactate concentration is reduced at similar absolute exercise intensities after a period of training. Moreover, the decreases found in the present study averaged around 30 to 35% during some stages of the grade-lactate test and endurance walk. These changes are greater than the 25 to 30% reductions found by Seals and co-workers (1984b) after a six month walking programme and greater also than the 18% reductions found by Hudson (1991) after a one year walking programme.

Ivy and colleagues (1980) have shown that the onset of blood lactate accumulation is strongly related to muscle respiratory capacity. Moreover, it has been demonstrated that the running speeds equivalent to various reference blood lactate concentrations correlate well with endurance performance (Farrell et al. 1979; Lafontaine et al. 1981; Londeree and Ames, 1975; Sjodin and Jacobs, 1981; Williams and Nute, 1983). Thus, in the present study, the reductions in submaximal blood lactate concentration are indicative of improvements in the oxidative capacity of skeletal muscle in the walking group. This in turn would suggest that the walkers had acquired an enhanced ability to perform endurance exercise.

Within the walking group there was a beneficial alteration in the oxygen uptake/blood lactate relationship during the grade-lactate test such that oxygen uptake at a given blood lactate concentration was higher post-training than pre-training (Figure 5.3). This adaptation to endurance exercise has been shown in other training studies involving both running (Hurley et al. 1984) and walking (Seals et al. 1984b). As a result of this changed relationship the oxygen uptake at which blood lactate concentration reached 2 mmol.L\(^{-1}\) increased significantly in the walking group by 11.6%, 14.9% and 6.5% after
three, six and 12 months respectively (Table 5.8). This provides further
evidence to support the conclusion that the respiratory capacity of skeletal
muscle was enhanced in the walking group. However, although the walkers
experienced an increase of around 10% in the relative intensity (%VO\(_2\)max)
attained at a blood lactate concentration of 2 mmol.l\(^{-1}\), their response did not
differ significantly from that of the control group in which corresponding values
increased by an average of 4% (Table 5.8). Thus, changes in blood lactate
concentration were not as great when expressed in relative terms as
compared to absolute terms.

As far as the time course for changes in blood lactate concentration is
concerned most of the decrease found in the walking group occurred within
the first three months. Although there were further reductions by six months
these were only small and by 12 months blood lactate concentrations had
risen again slightly. Thus, three months of brisk walking would seem to be
adequate to provoke significant adaptations in skeletal muscle and if the brisk
walking is continued for a longer period it appears to be a case of diminishing
returns in terms of further adaptation.

An examination of the blood lactate data from the endurance walk (Table 5.6)
would suggest that there was an improvement in the walkers' metabolic
responses to steady state exercise. At base line the walkers' blood lactate
concentrations at the end of the endurance walk had decreased by 2.7% (0.1
mmol.l\(^{-1}\)) from the values at five minutes. During subsequent tests the average
reduction was 17.1% (0.5 mmol.l\(^{-1}\)). For the control group, 20 minute blood
lactate concentrations at three, six and 12 months averaged only 3.9% (0.1
mmol.l\(^{-1}\)) lower than five minute concentrations. In the pilot study (Chapter
Three, Section 3.12) it will be recalled that blood lactate concentration tended
to increase rather than decrease during the endurance walk (Figure 3.8). The
differences between these two studies are possibly related to the different
relative exercise intensities at which the walks were conducted i.e. 80% of
VO\(_2\)max in the pilot study and 70% of predicted VO\(_2\)max in the walking study.
Nevertheless it was shown in the pilot study that those subjects who
experienced the smallest increases in blood lactate concentration between
minutes five and ten of the walk were likely to continue walking for the longest.
Therefore, the improved blood lactate response to steady state exercise which
occurred in the walking group during the brisk walking study may be taken as
evidence of improved endurance fitness.
The lowered concentrations of blood lactate in the walking group during submaximal exercise may have been related to a decreased rate of lactate production, an increased rate of lactate removal or a combination of these two. A decrease in the production rate of lactate during exercise would have resulted if there was a reduction in glycolysis. This would occur if fat metabolism was increased and some suggestion of this is provided by the small reductions in the $R$ values of the walkers after 12 months (Figure 5.7). It is also possible that glycolysis was not reduced but that a greater portion of the pyruvic acid produced during glycolysis was shunted into the mitochondria to be oxidised in the citric acid cycle. This would reduce the amount of pyruvic acid which was converted to lactic acid. Again this is feasible since endurance training has been shown to cause an increase in the size and number of mitochondria (Saltin and Gollnick, 1983). Donovan and Brooks (1983), however, have shown that the primary result of endurance training in rats is to increase the rate of lactate removal during exercise. This increased removal may be caused by an increased oxidation of lactate during exercise and an increased uptake of lactate by the liver and perhaps also non-exercising muscles for resynthesis back to glycogen (Brooks and Fahey, 1984).

Aside from the above factors some of the decrease in blood lactate concentration in the walking group and all of the decrease in blood lactate concentration in the control group may have been related to other factors which influence lactate production. Oxygen uptake, for example, was decreased during treadmill exercise and, as mentioned above, this reduction in oxygen consumption would indicate a reduced energy expenditure during exercise. It follows that glycolysis may also have been reduced during exercise due to the reduced energy requirement. It is also possible that as the study progressed the subjects experienced a diminished arousal response to exercise testing and thus a decreased adrenaline secretion. Adrenaline stimulates glycolysis in skeletal muscle and therefore if adrenaline secretion is decreased glycolysis will also be decreased.

The pattern of change in predicted VO$_2$max (ml.kg$^{-1}$min$^{-1}$) is at first sight slightly surprising (Table 5.9). Although VO$_2$max was elevated in the walking group by 2.5% and 3.1% after three and six months respectively, by 12 months VO$_2$max values were 2.5% lower than base-line values. These findings contradict with those of previous walking studies which have shown increases in VO$_2$max of between 9.7% and 27.8% after various periods of training.
Moreover, in the control group, VO\textsubscript{2}max values were decreased from baseline by 4.5% and 8.5% after six and 12 months respectively. These reductions are greater than those which might be expected to occur as a consequence of the ageing process alone i.e. less than 1% per year from the age of 25 onwards according to Astrand and Rodahl (1986).

When the changes in predicted VO\textsubscript{2}max are considered alongside the changes in oxygen uptake and heart rate they become easier to interpret. For the walkers the oxygen uptake/heart rate relationship remained relatively stable over the course of the study and by 12 months there was only a slight decrease in the oxygen uptake attained for a given heart rate. In the control subjects, however, there was a greater change in the oxygen uptake/heart rate relationship and by 12 months oxygen uptake was reduced dramatically for a given heart rate (Figure 5.1). The apparent decrease in VO\textsubscript{2}max in the control subjects then can be explained in the following way. Each subject’s VO\textsubscript{2}max was predicted using an age predicted maximum heart rate and the submaximal oxygen uptake/heart rate relationship from the grade-lactate treadmill test. Since oxygen uptake was reduced for a given heart rate after 12 months, the oxygen uptake which was predicted to occur upon the attainment of maximum heart rate was also reduced. This may also explain the apparent lack of change in VO\textsubscript{2}max in the walking group.

The previous comments highlight one of the problems in interpolating from submaximal data to predict maximal oxygen uptake values. In all likelihood the decreases in predicted VO\textsubscript{2}max in the control group were related primarily to the reductions in submaximal oxygen uptake which occurred as the study progressed and the actual VO\textsubscript{2}max values of the control subjects probably did not change a great deal. Similarly, although the changes in VO\textsubscript{2}max within the walking group were apparently small, actual increases may have been masked by the changes in submaximal oxygen consumption. Whatever the interpretation for the changes in VO\textsubscript{2}max, an important finding in the present study was the differential response between the walkers and controls over time as indicated by the results of the two way analysis of variance. This suggests that the brisk walking programme did have a beneficial influence on VO\textsubscript{2}max and the difference between the walkers and controls was 6.1% by the end of the study (initial percentage difference in the VO\textsubscript{2}max values of the
walkers and controls minus the final percentage difference in the VO$_{2}$max values of the walkers and controls).

To assess whether or not the volume or intensity of brisk walking determined the extent of change (from base-line values) in heart rate, blood lactate concentration and VO$_{2}$max relationships between these variables were examined. The average estimated miles of brisk walking per day were significantly correlated with the changes in heart rate at stages two, three and four of the six month grade-lactate test and at stages three and four of the 12 month grade-lactate test (range in $r = -0.31$ to -0.37). Similarly the changes in heart rate after 15 and 20 minutes of the endurance walk at six months and at all stages of the endurance walk at 12 months were significantly related to the average miles of brisk walking completed per day (range in $r = -0.31$ to -0.37). Only two significant correlations were obtained between the average miles of brisk walking per day and changes in blood lactate concentration and these were at minutes ten and 15 of the 12 month endurance walk ($r = -0.35$ and -0.32 respectively). Finally, the changes in VO$_{2}$max after 12 months correlated significantly with the average miles of brisk walking completed per day ($r = 0.35$). Training intensity (as assessed by the average %HR$_{max}$ attained during the track walk) did not correlate significantly with any of the changes in heart rate, blood lactate or VO$_{2}$max.

One other interesting finding was that changes in heart rate and blood lactate were related to some degree. Thus, during the endurance walk changes in heart rate and lactate concentration after three, six and 12 months were significantly correlated at every observation point i.e. five, ten, 15 and 20 minutes (range in $r = 0.43$ to 0.65). Similarly, changes in heart rate and lactate at stages two, three and four of the grade-lactate test were significantly related to each other after three, six and 12 months (range in $r = 0.31$ to 0.67).

The above correlations suggest a tendency for greater reductions in heart rate and blood lactate concentration and greater increases in VO$_{2}$max in those walkers who completed the greatest volume of brisk walking. Training intensity, on the other hand, appeared to have little or no influence on the extent of change in heart rate, blood lactate concentration and VO$_{2}$max. Finally, changes in heart rate and blood lactate concentration were related to some degree, suggesting that cardiovascular change was greatest in subjects who demonstrated the greatest metabolic change and vice versa.
After 12 months VE.\(\text{VO}_2\)\(^{-1}\) values were increased in the walking group by an average of 7.3% during the grade-lactate test. This change is in the opposite direction to that which might be expected since it would indicate that the walkers had to exhale a greater amount of air in order to utilise one litre of oxygen at the end of the study as compared to the beginning. When the values of the walking group are compared with those in the control group, however, they do suggest a beneficial influence of brisk walking since VE.\(\text{VO}_2\)\(^{-1}\) values increased in the control group by almost twice as much as they did in the walking group i.e. 13.9% (Figure 5.4).

In both the walking and control groups the increased VE.\(\text{VO}_2\)\(^{-1}\) values were related to the fact that oxygen uptake values were decreased during the treadmill test (Figure 5.5) without accompanying decreases in ventilation (Figure 5.6). It is difficult to explain why this should be. Taken at face value these results suggest that the subjects in both groups became more economical throughout the study in terms of oxygen consumption but that there were no equivalent adjustments in the rate of ventilation (although there was a small reduction in ventilation in the walking group). This is perplexing since it would be logical to expect that ventilatory adjustments would occur concurrently with changes in oxygen uptake. It is possible that errors occurred in the measurement of ventilation or oxygen uptake during the study and that this explains the above findings. There is no reason to suspect that this was the case, however, since reference gases were used to calibrate the oxygen analyser and these gases were in turn calibrated against a gold standard reference gas. Moreover, the dry gas meter was calibrated using a Tissot spirometer.

The non-protein respiratory exchange ratio (\(R\)) gives an indication of the individual contributions of fat and carbohydrate to the total energy yield during exercise. Lower \(R\) values indicate a greater fat oxidation and vice-versa (an \(R\) value of 0.7 indicates 100% fat oxidation while an \(R\) value of 1.0 indicates 100% carbohydrate oxidation). One of the major adaptations to running exercise is an increased ability to oxidise fat at a given absolute exercise intensity thus sparing muscle glycogen and delaying fatigue (Sjodin and Svedenhag, 1985). Wallington (1989) has also observed that trained subjects rely on fat metabolism to a greater extent than untrained subjects during submaximal treadmill walking. However, no differences in the \(R\) values of
walking and control subjects were evident in the one year training study of Hudson (1991).

In the present investigation there was a slight decrease in the R values of the walkers at some stages of the grade-lactate treadmill test after 12 months although the changes were small (Figure 5.7). In the control group, however, R values were increased after 12 months and there was a significant interaction between the walkers and controls at stages two, three and four of the grade-lactate test. The 12 month R values of the walkers were on average 2.7% lower than those of the controls. This indicates that the walking programme had a small but significant influence on the walkers ability to oxidise fat which in turn would suggest an improved potential for endurance exercise.

The reductions in PRE (Figure 5.8) which occurred in both the walking and control groups during the study are probably due simply to habituation. At the beginning of the study the test procedures were new to the subjects and some of the men expressed doubts as to their ability to complete the exercise tests. Once they had been through all of the testing procedures, however, the subjects knew that they were capable of completing the exercise tests and thus became more confident. The reduced PRE values which occurred after three, six and 12 months then may simply be a reflection of the subjects' increased self-confidence. The Mann-Whitney U test did not reveal any differences in the PRE values of the walkers and controls during the study, a finding consistent with that of other training studies which have measured PRE during walking exercise (Hudson, 1991; Santiago et al. 1987).

5.5 Conclusion

The findings presented in this Chapter demonstrate that the endurance fitness of the subjects in the training group was markedly improved by the brisk walking programme. Particularly impressive were the reductions in blood lactate concentration demonstrated by the walkers during standardised treadmill exercise. This would suggest that the primary benefit derived from the brisk walking programme was an enhanced metabolic capacity within skeletal muscle although there was evidence also of cardiovascular adaptation. Moreover, most of the changes occurred within the first three to six months of the training programme with the second part of the training year.
acting as a maintenance period. It appears that training volume rather than training intensity was the primary determinant of the extent of adaptation.
6 LIPIDS AND LIPOPROTEINS

This Chapter is divided into two main Sections. In the first Section the repeatability in serum lipid and lipoprotein measurement is assessed to give some indication of the precision with which these variables were quantified. In the second Section the effects of the brisk walking programme on lipid and lipoprotein metabolism are examined.

6.1 Repeatability in serum lipid and lipoprotein measurement

6.1.1 Introduction

The problems associated with the measurement of serum lipids and lipoproteins were discussed in Section 2.4 of Chapter Two. In this Section it was noted that biological variation accounts for the greatest portion of the total variability in serum lipid and lipoprotein measurement in most cases. To reduce biological variation and therefore total variation it is desirable that multiple samples be collected and that the mean value from these samples be adopted to represent the true value. Thus, in the brisk walking study, two samples were collected from each individual at each observation point. To examine the extent to which precision in measurement was improved by this procedure the agreement between the two samples was assessed.

6.1.2 Methods

The procedures for lipid and lipoprotein collection and analysis are described in Chapter Three (Sections 3.4 and 3.5) and in the Appendices. Two 20 ml venous blood samples were collected from each of the 65 subjects, not more than five days apart, at base line and after three, six and 12 months of the brisk walking study. Subjects completed a 12 hour overnight fast before blood samples were collected. The serum form the samples was stored at -70 °C until required for analysis to determine the concentrations of total cholesterol, HDL-C, triglyceride and apoproteins A-I and B.

Statistical analysis of the data was performed using a method to assess repeatability in measurement described by Bland (1987). This involved first plotting a scattergram of the differences between the first and second sample (i.e. sample 1 - sample 2) against the mean to establish whether the errors
depended on the value of measurement i.e. whether there were larger errors for larger values. An example of such a plot is shown in Figure 6.1. If the measurement error was not related to the value of measurement (which it did not appear to be in any of the cases examined) the next step involved the calculation of the error standard deviation. This was ascertained using the following formula:

\[
s = \sqrt{\frac{1}{2n} \sum (X - Y)^2}
\]

where \(s\) = error standard deviation; \(n\) = the number of subjects; \(X\) = sample 1 and \(Y\) = sample 2. Finally, the value below which the difference between the two measurements (sample 1 and sample 2) would lie with a probability of 95% was calculated as \(1.96 \times \sqrt{2s^2}\). This is subsequently referred to as the 95% difference.

Two tailed T tests were used to test for significant differences between samples 1 and 2 while the relationship between samples was examined using the Pearson Product Moment correlation coefficient.
Figure 6.1 Absolute difference plotted against the mean for paired total cholesterol values collected from 57 subjects at the start of the brisk walking study.

6.1.3 Results

Table 6.1 shows serum concentrations of each of the assessed variables. For each variable the sample 1 mean, sample 2 mean and overall mean are given at base line, three, six and 12 months together with the mean difference between samples 1 and 2. For a few subjects, only one sample was obtained at certain observation points and therefore these subjects were excluded from the analysis. Also included in Table 6.1 are the probability values resulting from two tailed T tests used to compare samples 1 and 2 and the coefficients of determination obtained from correlations between samples 1 and 2. As can be seen from the Table, of the 20 comparisons made there were three significant differences between the first and second samples. These were for triglyceride concentration after three months, for HDL-C concentration after three months and for Apo A-I concentration at base line. Finally, the Table shows the error standard deviation (s) and the greatest value which the first and second samples are likely to be separated by in 95% of cases (95% difference).
<table>
<thead>
<tr>
<th>Variable</th>
<th>Time</th>
<th>n</th>
<th>mean 1</th>
<th>mean 2</th>
<th>mean diff.</th>
<th>mean</th>
<th>P value</th>
<th>$r^2$</th>
<th>s</th>
<th>95% diff.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>BL</td>
<td>60</td>
<td>1.505</td>
<td>1.514</td>
<td>-0.008</td>
<td>1.510</td>
<td>0.903</td>
<td>0.742</td>
<td>0.376</td>
<td>1.042</td>
</tr>
<tr>
<td>(mmol.l⁻¹)</td>
<td>3M</td>
<td>57</td>
<td>1.478</td>
<td>1.314</td>
<td>0.164</td>
<td>1.396</td>
<td>0.023*</td>
<td>0.622</td>
<td>0.387</td>
<td>1.072</td>
</tr>
<tr>
<td></td>
<td>6M</td>
<td>61</td>
<td>1.577</td>
<td>1.567</td>
<td>0.010</td>
<td>1.572</td>
<td>0.893</td>
<td>0.694</td>
<td>0.418</td>
<td>1.157</td>
</tr>
<tr>
<td></td>
<td>12M</td>
<td>62</td>
<td>1.493</td>
<td>1.456</td>
<td>0.037</td>
<td>1.475</td>
<td>0.489</td>
<td>0.825</td>
<td>0.291</td>
<td>0.807</td>
</tr>
<tr>
<td>TC</td>
<td>BL</td>
<td>57</td>
<td>5.685</td>
<td>5.654</td>
<td>0.030</td>
<td>5.670</td>
<td>0.602</td>
<td>0.861</td>
<td>0.305</td>
<td>0.845</td>
</tr>
<tr>
<td>(mmol.l⁻¹)</td>
<td>3M</td>
<td>58</td>
<td>5.665</td>
<td>5.696</td>
<td>0.031</td>
<td>5.681</td>
<td>0.421</td>
<td>0.923</td>
<td>0.206</td>
<td>0.569</td>
</tr>
<tr>
<td></td>
<td>6M</td>
<td>61</td>
<td>5.705</td>
<td>5.744</td>
<td>0.039</td>
<td>5.725</td>
<td>0.377</td>
<td>0.917</td>
<td>0.242</td>
<td>0.673</td>
</tr>
<tr>
<td></td>
<td>12M</td>
<td>62</td>
<td>5.628</td>
<td>5.583</td>
<td>0.046</td>
<td>5.606</td>
<td>0.426</td>
<td>0.839</td>
<td>0.316</td>
<td>0.876</td>
</tr>
<tr>
<td>HDL-C</td>
<td>BL</td>
<td>59</td>
<td>1.404</td>
<td>1.421</td>
<td>0.107</td>
<td>1.413</td>
<td>0.134</td>
<td>0.932</td>
<td>0.063</td>
<td>0.175</td>
</tr>
<tr>
<td>(mmol.l⁻¹)</td>
<td>3M</td>
<td>54</td>
<td>1.390</td>
<td>1.420</td>
<td>0.030</td>
<td>1.405</td>
<td>0.023*</td>
<td>0.913</td>
<td>0.068</td>
<td>0.190</td>
</tr>
<tr>
<td></td>
<td>6M</td>
<td>56</td>
<td>1.249</td>
<td>1.262</td>
<td>0.013</td>
<td>1.256</td>
<td>0.373</td>
<td>0.900</td>
<td>0.078</td>
<td>0.215</td>
</tr>
<tr>
<td></td>
<td>12M</td>
<td>57</td>
<td>1.296</td>
<td>1.309</td>
<td>0.013</td>
<td>1.303</td>
<td>0.349</td>
<td>0.917</td>
<td>0.075</td>
<td>0.209</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>BL</td>
<td>59</td>
<td>1.472</td>
<td>1.380</td>
<td>0.091</td>
<td>1.426</td>
<td>0.002*</td>
<td>0.516</td>
<td>0.167</td>
<td>0.462</td>
</tr>
<tr>
<td>(g.l⁻¹)</td>
<td>3M</td>
<td>58</td>
<td>1.409</td>
<td>1.385</td>
<td>0.024</td>
<td>1.397</td>
<td>0.065</td>
<td>0.839</td>
<td>0.070</td>
<td>0.195</td>
</tr>
<tr>
<td></td>
<td>6M</td>
<td>61</td>
<td>1.409</td>
<td>1.424</td>
<td>0.015</td>
<td>1.417</td>
<td>0.257</td>
<td>0.801</td>
<td>0.073</td>
<td>0.202</td>
</tr>
<tr>
<td></td>
<td>12M</td>
<td>61</td>
<td>1.393</td>
<td>1.396</td>
<td>0.003</td>
<td>1.395</td>
<td>0.786</td>
<td>0.867</td>
<td>0.056</td>
<td>0.155</td>
</tr>
<tr>
<td>Apo B</td>
<td>BL</td>
<td>59</td>
<td>0.731</td>
<td>0.715</td>
<td>0.016</td>
<td>0.723</td>
<td>0.273</td>
<td>0.687</td>
<td>0.080</td>
<td>0.222</td>
</tr>
<tr>
<td>(g.l⁻¹)</td>
<td>3M</td>
<td>58</td>
<td>0.731</td>
<td>0.721</td>
<td>0.010</td>
<td>0.726</td>
<td>0.093</td>
<td>0.918</td>
<td>0.033</td>
<td>0.090</td>
</tr>
<tr>
<td></td>
<td>6M</td>
<td>61</td>
<td>0.757</td>
<td>0.760</td>
<td>0.003</td>
<td>0.759</td>
<td>0.650</td>
<td>0.924</td>
<td>0.036</td>
<td>0.098</td>
</tr>
<tr>
<td></td>
<td>12M</td>
<td>61</td>
<td>0.770</td>
<td>0.761</td>
<td>0.008</td>
<td>0.766</td>
<td>0.218</td>
<td>0.919</td>
<td>0.037</td>
<td>0.101</td>
</tr>
</tbody>
</table>

Table 6.1 Comparison of serum lipid and lipoprotein values from each of two samples obtained no more than five days apart from the same individual. s = error standard deviation. 95% diff. = the value below which the difference between the two measurements will lie with a probability of 95%. * indicates significance.
6.1.4 Discussion

An examination of Table 6.1 reveals that the sample 1 and sample 2 mean values were actually quite close in all cases. In fact, if these values are rounded up to one decimal place then there are only three cases where the paired means are not identical i.e. for triglyceride after three months, for HDL-C after six months and for Apo A-I at base line. On this basis then, it appears that there is fairly good agreement between samples 1 and 2. However, in three cases the difference between samples 1 and 2 was significant, as indicated in the Table. The greatest difference was seen for triglyceride at three months. Here the sample 2 mean was 11% lower than the sample 1 mean.

Although the agreement between the mean value of sample 1 and the mean value of sample 2 was generally good the picture is different when examining individual subject variation. This was especially true for the triglyceride measurements as revealed in the last two columns of Table 6.1. In the first of these columns the error standard deviation is shown. The error standard deviation, as calculated above, gives a value which is lower than that which would have been obtained if the standard deviation of the differences (between samples 1 and 2) had been calculated in the usual way. Nevertheless, the error standard deviation for triglyceride is still large and the differences between samples become even more apparent when examining the next column i.e. the 95% differences. The 95% differences average around 1.0 mmol.l⁻¹ for triglyceride. In other words, in 95% of cases the difference in triglyceride concentration between sample 1 and 2 is likely to be no greater than 1.0 mmol.l⁻¹. However, 1.0 mmol.l⁻¹ amounts to nearly 70% of the mean values for triglyceride and shows that the individual variation in triglyceride concentration between samples 1 and 2 was large in many cases.

The 95% differences for total cholesterol, HDL-C and apoproteins A-I and B were much smaller than they were for triglyceride. If, for each of these four variables, the 95% difference is expressed as a percentage of the mean of samples 1 and 2, the following average percentages are gained: 13.1%; 14.8%; 18.0% and 17.3% for total cholesterol, HDL-C and apoproteins A-I and B respectively. This reveals that in all four cases the agreement between samples 1 and 2 was much better than it was for triglyceride concentration, while the total cholesterol and HDL-C measurements demonstrated the best
precision. This last point is also clearly indicated by the coefficient of determination values shown in Table 6.1. All the $r^2$ values for total cholesterol and HDL-C are above 0.8 with lower values being obtained for triglyceride and apoproteins A-I and B.

6.1.5 Conclusion

The individual variations between the first and second samples were large in the case of triglyceride, though there was a much better agreement between samples for the other variables assessed, particularly total cholesterol and HDL-C. These findings support those from previous studies which have shown that biological variation is greater for triglyceride concentration than than it is for the concentrations of other lipids and lipoproteins (Bookstein et al. 1990; Mogadam et al. 1990; Demacker et al. 1982). Nevertheless, there were significant differences between the first and second samples for HDL-C and Apo A-I concentration in addition to triglyceride concentration. Therefore, these findings suggest that measurement precision was improved by the procedure of collecting and analysing two samples from each subject and in some cases this procedure was essential.
6.2 The effects of brisk walking on serum lipids and lipoproteins

6.2.1 Introduction

It is clear from the discussion in Chapter Five that the brisk walking programme had a beneficial influence on the endurance fitness of the walking group. This was demonstrated by the profound reductions in blood lactate concentration which occurred during treadmill exercise and also by the indications of cardiovascular adaptation. The following Section examines the effects of these changes on serum lipids and lipoproteins and in particular on HDL-C which responds more consistently to exercise training than other lipoprotein variables.

6.2.2 Methods

Two 20 ml venous blood samples were collected from each subject, not more than five days apart, at baseline, three, six and 12 months. A portion of this blood was used immediately for the measurement of haemoglobin concentration and haematocrit while the concentration of total cholesterol, HDL-C, triglyceride, apoproteins A-I and B and Lp(a) was determined later from frozen serum. Additionally, LDL-C and VLDL-C were estimated using the Friedewald formula (Friedewald et al. 1972). Measurement of HDL-C subfractions by precipitation proved unreliable and thus no information for HDL2-C or HDL3-C is presented here. The procedures employed in the measurement of the above variables are described in more detail in Sections 3.4 and 3.5 and in the Appendices.

Measurements of body density were made at the beginning and end of the study by means of hydrostatic weighing and from these the percentage of body fat was determined for each subject (Section 3.9).

Energy intake was assessed at baseline and six and 12 months into the study via the seven day weighed food inventory technique (Section 3.8).

As in the previous Chapter the responses of the walkers and controls were compared using two way analysis of variance for repeated measures and relationships between variables were examined using the Pearson Product
Moment correlation coefficient. Unless otherwise stated the number of subjects in each group is 42 and 23 for walkers and controls respectively.

6.2.3 Results

Tables 6.2 and 6.3 display base-line, three, six and 12 month values for all of the lipids and lipoproteins determined during the study. Although there were significant changes through the year in HDL-C, the ratio of total cholesterol to HDL-C, LDL-C, Apo B and Lp(a) concentration, no significant interactions were obtained between the walkers and controls for any of these variables.

Changes in body composition during the study are shown in Table 6.4 while the values for energy intake are displayed in Table 6.5. Interactions between walkers and controls were not significant for any of these variables although the body composition measures did vary significantly through the year.

Haemoconcentration or haemodilution will affect the concentrations of blood lipids and lipoproteins. To ensure that these were accounted for haemoglobin and haematocrit were measured during the study and the results are shown in Table 6.6. Both the walkers and the controls experienced a small but significant decrease in haemoglobin concentration as the study progressed. There were also significant changes in haematocrit but again these changes were small. No significant interactions were found when comparing the haemoglobin or haematocrit values of the walkers and controls. Moreover, serum lipoprotein concentrations were not adjusted to compensate for changes in haematocrit.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Base line</th>
<th>3 months</th>
<th>6 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mmol.L(^{-1}))</td>
<td>W</td>
<td>5.7 ± 0.2</td>
<td>5.8 ± 0.2</td>
<td>5.8 ± 0.2</td>
<td>5.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>5.7 ± 0.3</td>
<td>5.6 ± 0.2</td>
<td>5.7 ± 0.3</td>
<td>5.6 ± 0.2</td>
</tr>
<tr>
<td>HDL-C (mmol.L(^{-1}))</td>
<td>W</td>
<td>1.4 ± 0.1</td>
<td>1.4 ± 0.0</td>
<td>1.3 ± 0.1</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1.4 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>W</td>
<td>4.1 ± 0.2</td>
<td>4.3 ± 0.2</td>
<td>4.7 ± 0.2</td>
<td>4.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>4.3 ± 0.3</td>
<td>4.5 ± 0.3</td>
<td>5.0 ± 0.3</td>
<td>4.7 ± 0.3</td>
</tr>
<tr>
<td>LDL-C (mmol.L(^{-1}))</td>
<td>W</td>
<td>3.6 ± 0.1</td>
<td>3.8 ± 0.2</td>
<td>3.8 ± 0.1</td>
<td>3.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>3.6 ± 0.3</td>
<td>3.6 ± 0.2</td>
<td>3.7 ± 0.2</td>
<td>3.6 ± 0.2</td>
</tr>
<tr>
<td>VLDL-C (mmol.L(^{-1}))</td>
<td>W</td>
<td>0.7 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.7 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>Apo A-I (g.L(^{-1}))</td>
<td>W</td>
<td>1.4 ± 0.0</td>
<td>1.4 ± 0.0</td>
<td>1.4 ± 0.0</td>
<td>1.4 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1.4 ± 0.1</td>
<td>1.4 ± 0.0</td>
<td>1.4 ± 0.0</td>
<td>1.4 ± 0.0</td>
</tr>
<tr>
<td>Apo B (g.L(^{-1}))</td>
<td>W</td>
<td>0.7 ± 0.0</td>
<td>0.7 ± 0.0</td>
<td>0.8 ± 0.0</td>
<td>0.8 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.7 ± 0.0</td>
<td>0.7 ± 0.0</td>
<td>0.8 ± 0.0</td>
<td>0.8 ± 0.0</td>
</tr>
</tbody>
</table>

Table 6.2 Serum lipoprotein parameters for walkers and controls during the brisk walking study (mean ± SEM). No significant interactions.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Base line</th>
<th>3 months</th>
<th>6 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG (mmol.l⁻¹)</td>
<td>W</td>
<td>1.3 (1.1 - 1.6)</td>
<td>1.2 (1.0 - 1.5)</td>
<td>1.3 (1.1 - 1.5)</td>
<td>1.3 (1.1 - 1.5)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1.4 (1.1 - 1.8)</td>
<td>1.4 (1.1 - 1.8)</td>
<td>1.5 (1.1 - 1.9)</td>
<td>1.4 (1.1 - 1.8)</td>
</tr>
<tr>
<td>Lp(a) (mg.dl⁻¹)</td>
<td>W</td>
<td>13.2 (8.5 - 20.6)</td>
<td>-</td>
<td>-</td>
<td>16.8 (11.1 - 25.5)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>7.1 (3.6 - 14.1)</td>
<td>-</td>
<td>-</td>
<td>13.3 (8.1 - 21.8)</td>
</tr>
</tbody>
</table>

Table 6.3 Serum triglyceride (TG) and Lp(a) concentrations from the brisk walking study for walkers and controls. Values are antilogs of the mean and 95% confidence intervals of log data. No significant interactions.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Base line</th>
<th>3 months</th>
<th>6 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (kg)</td>
<td>W</td>
<td>79.1 ± 1.5</td>
<td>78.4 ± 1.5</td>
<td>79.2 ± 1.5</td>
<td>78.9 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>78.2 ± 2.5</td>
<td>77.6 ± 2.5</td>
<td>78.8 ± 2.5</td>
<td>78.9 ± 2.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>W</td>
<td>25.4 ± 0.4</td>
<td>25.2 ± 0.4</td>
<td>25.4 ± 0.4</td>
<td>25.4 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>24.9 ± 0.7</td>
<td>24.8 ± 0.7</td>
<td>25.1 ± 0.7</td>
<td>25.1 ± 0.7</td>
</tr>
<tr>
<td>%fat</td>
<td>W</td>
<td>28.8 ± 0.8</td>
<td>-</td>
<td>-</td>
<td>27.7 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>29.9 ± 1.5</td>
<td>-</td>
<td>-</td>
<td>29.6 ± 1.6</td>
</tr>
</tbody>
</table>

Table 6.4 Body mass, body mass index (BMI) and the percentage of body fat for walkers and controls during the brisk walking study (mean ± SEM). No significant interactions.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Base line</th>
<th>6 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (MJ)</td>
<td>W</td>
<td>10.7 ± 0.3</td>
<td>10.4 ± 0.3</td>
<td>10.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>10.7 ± 0.3</td>
<td>11.0 ± 0.4</td>
<td>10.9 ± 0.5</td>
</tr>
</tbody>
</table>

Table 6.5 Daily energy intake for walkers and controls during the brisk walking study (mean ± SEM). No significant interaction.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Base line</th>
<th>3 months</th>
<th>6 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g.100 ml⁻¹)</td>
<td>W 15.0 ± 0.1</td>
<td>14.9 ± 0.2</td>
<td>14.8 ± 0.2</td>
<td>14.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>C 15.2 ± 0.1</td>
<td>15.1 ± 0.1</td>
<td>15.1 ± 0.2</td>
<td>14.8 ± 0.1</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>W 45.0 ± 0.3</td>
<td>44.3 ± 0.5</td>
<td>44.9 ± 0.4</td>
<td>44.3 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>C 45.5 ± 0.4</td>
<td>44.9 ± 0.3</td>
<td>45.9 ± 0.4</td>
<td>44.8 ± 0.4</td>
</tr>
</tbody>
</table>

Table 6.6 Haemoglobin concentration and haematocrit for walkers and controls during the brisk walking study (mean ± SEM). No significant interactions.
6.2.4 Discussion

From Tables 6.2 and 6.3 it can be seen that the random allocation procedure was successful in matching the walking and control groups in terms of serum lipid and lipoprotein concentrations. Considering the volume of training completed by the walking group it is surprising that there were no significant interactions between the two groups for any of the lipoprotein variables or for the body composition and energy intake measures.

The fact that there was no hint of change in HDL-C in this investigation is particularly surprising, bearing in mind the findings from many previous studies. Possible reasons for the lack of change in HDL-C include one or more of the following: 1: an insufficient volume of training; 2: an insufficient intensity of training; 3: the lack of an associated change in body weight/body fatness and 4: the base-line concentrations of serum lipids and lipoproteins. It may be, for example, that the initial concentrations of HDL-C were sufficiently high that further change was unlikely. These points will be taken in turn in the following discussion.

Before commencing this discussion it is worth noting that the initial lipid and lipoprotein concentrations of the men in this study compare favourably with those of the typical British male. For example, mean total cholesterol concentration at base line was 5.7 mmol.l⁻¹ for the walkers and controls (Table 6.2). Although this is higher than desirable (<5.2 mmol.l⁻¹) according to the British Hyperlipidaemia Association (Shepherd et al. 1987) it is still lower than the values found by Mann and colleagues (1988) for British males aged 40 to 59 years (between 6.0 and 6.1 mmol.l⁻¹). It is lower also than the value found by Pocock and colleagues (1986) in the British Regional Heart Study. In this study, data on over 7000 asymptomatic men aged 40 to 59 years was collected and the mean total cholesterol concentration of these men was 6.3 mmol.l⁻¹. Furthermore, the serum triglyceride concentrations of the walkers and controls at base line (Table 6.3) were lower than the mean triglyceride concentration of the British males aged 45 to 59 years in the study of Mann and colleagues (1988) i.e. 1.9 mmol.l⁻¹. Mann and colleagues (1988) did not log transform their data, however, which may partly explain the differences. Finally, the mean HDL-C concentration for both the walking and control groups at base line was 1.4 mmol.l⁻¹. This is higher than the mean concentration of HDL-C found by Pocock and colleagues (1986) in the
asymptomatic men examined in the British Regional Heart Study i.e. 1.1 mmol.l⁻¹. Thus, the subjects in the brisk walking study exhibited lipid and lipoprotein profiles which compare favourably with aged matched, asymptomatic, British males.

In his review on exercise training and lipoproteins Haskell (1986) proposes that an elevation in HDL-C will frequently occur when exercise energy expenditure is increased by around 5 MJ (1200 kcal) per week in previously sedentary healthy persons. Moreover, some studies have shown significant changes in HDL-C concentrations with increases in energy expenditure which are probably less than 5 MJ per week (Hardman et al. 1989a; Marti et al. 1990; Stein et al. 1990). In Chapter Four (Section 4.8.1) it was estimated that the energy expenditure of horizontal walking for the males in the brisk walking study would have been approximately 93 kcal per mile assuming a walking speed of 4 mph. Moreover, based on the average walking speed and the average time spent walking it was estimated that the walkers completed an average of 14.1 miles of brisk walking per week (Section 4.8.3). This gives an estimated weekly energy expenditure of around 5.5 MJ (1300 kcal) which, if anything, is an underestimate as the average walking speed of the training group was over 4 mph. Nevertheless, this estimate exceeds the value of 5.0 MJ suggested by Haskell (1986) as being sufficient to stimulate increases in HDL-C concentration. It is also greater than the weekly energy expenditures attained during exercise in many of the training studies mentioned above, yet no changes in HDL-C concentration were observed.

Wood and colleagues (1984) argue that a certain threshold of exercise must be reached before beneficial changes in lipoproteins occur. Their findings suggest that this threshold is around eight to ten miles of running per week (Williams et al. 1982; Wood et al. 1983). Other authors also propose the idea of a threshold effect, including Superko (1991) in a recent review. This issue was addressed in the present investigation by re-examining the HDL-C data after excluding those subjects who completed less than an average of ten miles of brisk walking per week. This process was subsequently repeated two more times by removing subjects who had completed less than 15 miles of brisk walking per week and finally less than 20 miles of brisk walking per week from the analysis. The results are shown below (Table 6.7).
Table 6.7 Miles per week of brisk walking and HDL-C concentration (mmol.l⁻¹) for the walking group as a whole and for subgroups of walkers comprising those completing above 10, 15 and 20 miles per week. For reference purposes control values for HDL-C are also included. Values are mean ± SEM.
As can be seen from Table 6.7 the amount of brisk walking performed each week had no influence on the change in HDL-C concentration. In all groups HDL-C concentration was decreased from base-line values by around 7% after 12 months. Furthermore, there were no significant correlations within the walking group as a whole between either the minutes or miles of brisk walking performed during the study and changes in HDL-C concentration after three, six or 12 months. This finding conflicts with that of some previous studies which have shown significant correlations between training volume and changes in HDL-C (Marti et al. 1990; Wood et al. 1983; Wood et al. 1988).

The lack of change in HDL-C in the present study probably cannot be attributed solely to an inadequate volume of training because previous studies have shown changes in HDL-C with somewhat smaller volumes of training (Marti et al. 1990; Stein et al. 1990; Wood et al. 1983; Wood et al. 1988). It is possible, however, that training volume and intensity interact and that a larger volume of low intensity training would have had an effect on HDL-C. There is also the possibility that a longer period of training would have provoked changes in HDL-C concentration. This suggestion is supported by the study of Wood and associates (1983) where correlations between reported miles run and changes in HDL-C concentration only became significant after 12 months.

The question of exercise intensity is a difficult one to address because in many longitudinal studies training intensity is not closely monitored and therefore can only be estimated approximately. Even when training intensity is measured precisely there are often factors which make comparison with other studies difficult. For example, there may be differences in the mode and frequency of training and also in the characteristics of the subject groups studied. In other cases the way in which exercise intensity is measured and defined makes cross comparison between studies difficult. Marti and co-workers (1990), for example, used a training intensity equivalent to 85% of the heart rate attained at the anaerobic threshold in their study while Sasaki and associates (1987) prescribed their training on the basis of running velocity at the blood lactate threshold.

In the present study exercise intensity was assessed during the track walks and averaged 68% of HRmax (range = 53% to 88%) which corresponds to approximately 57% of VO2max (ACSM, 1991). It was assumed that the exercise intensity attained during the track walks was equivalent to the
intensity at which the walkers performed their brisk walking. It is possible that this intensity was too low to provoke changes in HDL-C concentration since most studies showing increases in HDL-C concentration with exercise training have employed intensities equal to or above 70% of HRmax (Faria and Faria, 1991; Kiens et al. 1980; Mendoza et al. 1991; Stein et al. 1990; Stubbe et al. 1983; Thompson et al. 1988; Wood et al. 1983). However, very few studies have examined the effects of lower intensities of exercise on serum lipids and even when this has been attempted the results have not always been conclusive. Gaesser and Rich (1984) and Savage and co-workers (1986), for example, have compared the effects of low (40% to 45% of VO₂max) and high (75% to 85% of VO₂max) intensity exercise on serum lipids. In both studies, however, there were no changes in HDL-C concentration in either condition.

Ward and colleagues (1989) found no significant changes in HDL-C concentration in a group of overweight men who followed a walking programme (50 to 55% of HRmax) over a 16 week period. Brisk walking has, however, been shown to increase HDL-C concentration in women (Hardman et al. 1989a). In the latter study the average exercise intensity was 60% of VO₂max, which is similar to that of the present study, and HDL-C was increased by 27% over a one year period. It is possible that the conflicting results of these two studies may be attributed to a sex difference in the lipoprotein response to exercise training. It has been shown previously, for example, that exercise training at 70% of HRmax is sufficient to increase HDL-C concentration in women but not in men (Hill et al. 1989). However, in this same study total cholesterol concentration was reduced significantly in men but not in women demonstrating how confusing many of the findings from these studies can be.

In an attempt to take a closer look at the effects of exercise intensity on HDL-C the data from the present study was re-examined in the following way. Subjects were removed from the data bank in a step wise fashion starting with those who exercised at an intensity which was below 60% of HRmax. Next those subjects who exercised at an intensity which was below 70% of HRmax were removed from the data bank and finally those subjects who exercised at an intensity which was below 80% of HRmax were deleted from the data bank. High density lipoprotein cholesterol concentration was re-calculated in each case and the results are displayed in Table 6.8.
### Table 6.8

<table>
<thead>
<tr>
<th>Group</th>
<th>Controls</th>
<th>All Walkers</th>
<th>&gt;60% HRmax</th>
<th>&gt;70% HRmax</th>
<th>&gt;80% HRmax</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>23</td>
<td>42</td>
<td>30</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>%HRmax: mean</td>
<td>67.4 ± 1.6</td>
<td>67.6 ± 1.3</td>
<td>71.5 ± 1.3</td>
<td>79.2 ± 1.5</td>
<td>84.4 ± 1.3</td>
</tr>
<tr>
<td>%HRmax: range</td>
<td>52.5 - 85.0</td>
<td>52.5 - 88.1</td>
<td>63.0 - 88.1</td>
<td>70.3 - 88.1</td>
<td>82.7 - 88.1</td>
</tr>
<tr>
<td>mile.wk(^{-1}): mean</td>
<td>-</td>
<td>14.1 ± 0.7</td>
<td>13.7 ± 0.8</td>
<td>17.1 ± 0.9</td>
<td>15.6 ± 0.8</td>
</tr>
<tr>
<td>mile.wk(^{-1}): range</td>
<td>-</td>
<td>5.5 - 23.3</td>
<td>5.5 - 21.5</td>
<td>13.8 - 21.5</td>
<td>13.8 - 17.8</td>
</tr>
<tr>
<td>HDL-C BL</td>
<td>1.4 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>HDL-C 3M</td>
<td>1.3 ± 0.1</td>
<td>1.4 ± 0.0</td>
<td>1.5 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>HDL-C 6M</td>
<td>1.2 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>HDL-C 12M</td>
<td>1.3 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.4 ± 0.1</td>
</tr>
</tbody>
</table>

Table 6.8 Exercise intensity (%HRmax) and HDL-C concentration (mmol.L\(^{-1}\)) for the walking group as a whole and for subgroups of walkers training at intensities above 60%, 70% and 80% of HRmax. For reference purposes the miles per week of brisk walking completed by each group is included together with the control group values for HDL-C. Values are mean ± SEM.
As can be seen from Table 6.8 there is a trend for increased concentrations of HDL-C in the groups exercising at higher intensities. However, the pattern of change over the course of the study is similar regardless of exercise intensity, that is, for each group shown in Table 6.8 HDL-C concentration is lower after 12 months than it is at base line. Furthermore, correlations for the whole group of walkers between training intensity, as assessed by %HRmax during the track walk, and the changes in HDL-C concentration after three, six and 12 months were all low (r = 0.09, 0.34 and 0.19 respectively), although the correlation at 6 months was significant. These results do not suggest that the lack of change in HDL-C concentration during this study was due solely to an insufficient training intensity.

In many, but not all longitudinal studies changes in HDL-C concentration after a period of exercise training have occurred concomitantly with changes in body weight/body fat (Despres et al. 1990; Hespel et al. 1988b; Marti et al. 1990; Sasaki et al. 1987; Wood et al. 1985; Wood et al. 1988). Although there were reductions in both body weight and body fat in some of the walkers in the present study, values for the walking group as a whole remained largely unchanged (Table 6.4). Thus, it is possible that the inability of the exercise programme to increase HDL-C was due to a lack of change in body fatness. However, within the walking group changes in percent body fat did not correlate significantly with changes in HDL-C concentration after 12 months (r = 0.03). This would not support the argument that the lack of change in HDL-C was due to the lack of change in body fat.

When the individual data were examined more thoroughly it was found that 23 of the 42 walkers experienced a reduction in body fat during the study. However, HDL-C concentration was not increased even in these subjects after 12 months (Table 6.9). The relationship between changes in percent body fat and changes in HDL-C concentration after 12 months was not significant in this group either (r = 0.05). Moreover, in one individual body weight was decreased by 5.7% (from 102.3 kg to 96.5 kg) and body fat was decreased by 24.9% (from 41.0% to 30.8%) by the end of the study and yet HDL-C concentration was still 9.1% lower after 12 months than it had been at base line (base-line HDL-C = 1.1 mmol.l⁻¹; 12 months HDL-C = 1.0 mmol.l⁻¹). Therefore, an increase in HDL-C concentration is not an inevitable consequence of fat loss in some individuals.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Observation</th>
<th>Mean ± SEM</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>%fat</td>
<td>Base line</td>
<td>30.2 ± 1.2</td>
<td>21.0 - 41.0</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>27.2 ± 1.0</td>
<td>19.3 - 35.7</td>
</tr>
<tr>
<td></td>
<td>Net change</td>
<td>-3.0 ± 0.5</td>
<td>-10.3 - -0.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>Base line</td>
<td>79.1 ± 2.2</td>
<td>60.0 - 102.3</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>78.1 ± 1.9</td>
<td>59.6 - 96.5</td>
</tr>
<tr>
<td></td>
<td>Net change</td>
<td>-1.0 ± 0.5</td>
<td>-5.9 - 2.7</td>
</tr>
<tr>
<td>HDL-C (mmol.l⁻¹)</td>
<td>Base line</td>
<td>1.5 ± 0.1</td>
<td>0.8 - 2.4</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>1.4 ± 0.1</td>
<td>0.8 - 2.2</td>
</tr>
<tr>
<td></td>
<td>Net change</td>
<td>-0.1 ± 0.0</td>
<td>-0.6 - 0.2</td>
</tr>
</tbody>
</table>

Table 6.9 Percentage body fat, body weight and HDL-C concentration in 23 walkers who experienced a net reduction in their percentage body fat by the end of the brisk walking study.
It has been shown previously that the extent of change in HDL-C concentration with training is related to pretraining values, i.e. those subjects who have the lowest levels of HDL-C initially are likely to experience the greatest changes in HDL-C after a period of training (Sutherland and Woodhouse, 1980). Moreover, the initial HDL-C concentration of the walking group in the present study was 1.4 mmol.l\(^{-1}\). This is higher than the base-line concentrations of HDL-C found in many other studies where there are subsequent increases in HDL-C with training. For example, in the studies of Hardman and colleagues (1989a), Marti and co-workers (1990), Thompson and associates (1988) and Wood and colleagues (Wood et al. 1983; Wood et al. 1988) base-line HDL-C concentrations ranged from between 1.0 and 1.3 mmol.l\(^{-1}\). It is true that the different precipitation procedures used in these studies may partly account for the differences in HDL-C concentration. However, the maximum difference between procedures should not be more than around 0.1 mmol.l\(^{-1}\) and if anything the manganese chloride - sodium heparin precipitation procedure, used in the present study, underestimates rather than overestimates HDL-C concentration (Valiance, personal communication). Thus, base-line values in the present study may actually have been slightly higher than 1.4 mmol.l\(^{-1}\).

In view of the fact that the base-line concentration of HDL-C may have had an impact on the subsequent HDL-C response to exercise training, the data from the present study was re-examined by grouping the subjects according to their initial HDL-C concentrations as shown in Table 6.10.
<table>
<thead>
<tr>
<th>Group</th>
<th>Controls</th>
<th>All Walkers</th>
<th>HDL &lt;1.8 mmol.l(^{-1})</th>
<th>HDL &lt;1.5 mmol.l(^{-1})</th>
<th>HDL &lt;1.2 mmol.l(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>23</td>
<td>42</td>
<td>35</td>
<td>26</td>
<td>10</td>
</tr>
<tr>
<td>%HRmax: mean</td>
<td></td>
<td>67.6 ± 1.3</td>
<td>67.8 ± 1.5</td>
<td>65.3 ± 1.5</td>
<td>65.8 ± 2.7</td>
</tr>
<tr>
<td>%HRmax: range</td>
<td></td>
<td>52.5 - 88.1</td>
<td>55.2 - 88.1</td>
<td>55.2 - 82.9</td>
<td>57.4 - 79.7</td>
</tr>
<tr>
<td>mile.wk(^{-1}): mean</td>
<td></td>
<td>14.1 ± 0.7</td>
<td>14.1 ± 0.8</td>
<td>14.0 ± 1.0</td>
<td>14.9 ± 1.6</td>
</tr>
<tr>
<td>mile.wk(^{-1}): range</td>
<td></td>
<td>5.5 - 23.3</td>
<td>5.5 - 23.3</td>
<td>5.5 - 23.3</td>
<td>6.7 - 21.0</td>
</tr>
<tr>
<td>HDL-C BL</td>
<td>1.4 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.3 ± 0.0</td>
<td>1.2 ± 0.0</td>
<td>1.1 ± 0.0</td>
</tr>
<tr>
<td>HDL-C 3M</td>
<td>1.3 ± 0.1</td>
<td>1.4 ± 0.0</td>
<td>1.3 ± 0.0</td>
<td>1.3 ± 0.0</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>HDL-C 6M</td>
<td>1.2 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>1.2 ± 0.0</td>
<td>1.1 ± 0.0</td>
<td>0.9 ± 0.0</td>
</tr>
<tr>
<td>HDL-C 12M</td>
<td>1.3 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>1.0 ± 0.0</td>
</tr>
</tbody>
</table>

Table 6.10 HDL-C concentration (mean ± SEM) for the walking group as a whole and for subgroups of walkers whose base-line HDL-C concentrations were below 1.8, 1.5 and 1.2 mmol.l\(^{-1}\). The estimated miles per week of brisk walking and training intensity (%HRmax) of each group are also given together with the HDL-C concentration of the control group.
It is evident from Table 6.10 that base-line HDL-C concentrations had little influence on subsequent changes in HDL-C concentration with training. In all subgroups the tendency was for HDL-C concentration to decrease over the training period. Moreover, although there was a significant correlation for the walking group as a whole between base-line HDL-C concentration and the change in HDL-C concentration after three months of training ($r = -0.44$), a similar relationship was observed in the control group ($r = -0.43$). It is possible that some of the control subjects were tempted to do some training and that this explains the latter correlation. However, this seems unlikely because the changes in blood lactate concentration, presented in Chapter Five, were much greater in the walkers than in the controls. Moreover, correlations between initial HDL-C concentration and the changes in HDL-C concentration after six and 12 months were not significant for either the walking or control groups.

On the basis of the previous discussion then there is no clear cut answer as to why HDL-C concentration did not change with training. There is no strong evidence linking the lack of change in HDL-C to an inadequate volume or intensity of training, to the absence of any change in body fat percentage or to the base-line concentrations of HDL-C. However, it is possible that a combination of two or more of these factors may explain the lack of change in HDL-C.

One other possible explanation as to why HDL-C concentration was not significantly increased in the walking group during this study relates to energy balance. In their one year training study Wood and colleagues (1983) found that changes in energy intake were positively correlated with changes in HDL-C. In the present study there was no evidence that energy intake (assessed via the weighed food inventory technique) had changed after six or 12 months (Table 6.5). Bearing in mind the presumed increase in energy expenditure and the fact that there were no significant changes in body fatness in the walkers this finding is surprising. It may be that the walkers compensated for the increased energy expenditure during the walking by reducing their energy expenditure during the rest of the day. If this is true then it is possible that the lack of change in energy intake partly explains the HDL-C findings. However, other studies have shown increases in HDL-C with training in the absence of any changes in body fatness and energy intake (Hudson, 1991).
while there was a trend for a slight increase in triglyceride concentration in the control group, the difference between the two groups was not significant (Table 6.3). This finding conflicts with some previous reports of low triglyceride concentrations in trained subjects (Thompson et al. 1988; Weintraub et al. 1989; Wood et al. 1988). Other studies, however, have failed to show significant changes in triglyceride concentration after a period of exercise training even when increases in HDL-C concentration have occurred (Despres et al. 1990; Marti et al. 1990).

The significance of apoproteins A-I and B for CHD was discussed in the literature review (Section 2.3). Here it was mentioned that Apo A-I is the major protein present in HDL while Apo B is virtually the sole protein component of LDL. Moreover, it was noted that in some cases the discriminative powers of these apoproteins in detecting CHD have proved superior to those of HDL-C and LDL-C. Due to their significance, apoproteins A-I and B were measured in the brisk walking study.

Apoprotein A-I concentrations remained extremely stable throughout the study in both the walking and control groups at 1.4 g.l\(^{-1}\). Apoprotein B concentrations increased in both groups after six months from 0.7 g.l\(^{-1}\) to 0.8 g.l\(^{-1}\) and remained at this level at 12 months (Table 6.2). There was no difference in the response of the walkers and the controls for either Apo A-I or Apo B. These findings conflict with those of several cross-sectional studies which have reported higher concentrations of Apo A-I (Herbert et al. 1984; Nagao et al. 1988; Sady et al. 1988; Thompson et al. 1983) and Apo B (Giada et al. 1988) in trained as compared to sedentary subjects. However, the results from longitudinal studies have not been as consistent as those from observational studies and many have found, as in the present study, that concentrations of Apo A-I (Despres et al. 1990; Hespel et al. 1988a; Iltis et al. 1984; Marti et al. 1990; Thompson et al. 1988; Weintraub et al. 1989; Wood et al. 1983) and Apo B (Despres et al. 1988; Iltis et al. 1984; Marti et al. 1990; Weintraub et al. 1989; Wood et al. 1983) are unchanged with training.

One of the most novel aspects of this study was its examination of the effects of brisk walking on Lp(a) concentration. As mentioned in Chapter Two (Section 2.3.12) individuals with elevated Lp(a) concentrations have an increased risk of developing CHD. This relationship is independent of other risk factors (Dahlen et al. 1986). The metabolic role Lp(a) is largely unknown but is
thought to be related to either blood clotting, lipoprotein metabolism or both. There has recently been an upsurge of interest in Lp(a) due to the finding that the amino acid sequence of apolipoprotein(a) (present in Lp(a)) is almost identical to that of plasminogen (Lawn, 1992). Plasminogen is a precursor of plasmin which is involved in the dissolution of fibrin and therefore, blood clots. The implication of this finding is that Lp(a) may interfere with fibrinolysis and in this way be related to the long term development of CHD and/or the final acute event precipitating a heart attack.

The concentration of Lp(a) within an individual is largely genetically determined (Boerwinkle, 1992) and varies over an enormous range. Dahlen (1988), for example, has observed values of between 0 and 130 mg.dl\(^{-1}\). In the present study values ranged from below 5 mg.dl\(^{-1}\) to 79 mg.dl\(^{-1}\) when the walking and control groups were combined. Moreover, at base line 21 subjects had Lp(a) concentrations which were above 30 mg.dl\(^{-1}\) and after 12 months 25 subjects had Lp(a) concentrations which were above 30 mg.dl\(^{-1}\). According to Rees and colleagues (1990) these subjects have a relative risk of CHD which is two fold greater than those with lower concentrations of Lp(a). The mean concentrations of total cholesterol and LDL-C were also slightly (0.2 to 0.4 mmol.l\(^{-1}\)) higher in these subjects than the overall group means but the other lipid and lipoprotein concentrations of these subjects were similar to the group means.

There is very little data on the effects of environmental factors on Lp(a) concentration. Dietary changes and drug treatments which have previously been shown to be effective in lowering other lipid risk factors have little or no effect on Lp(a) concentration (Lawn, 1992; Loscalzo, 1990). Even less is known regarding the effects of such factors as weight loss and exercise. In a study by Sonnichsen and colleagues (1990) weight loss by dieting led to an average 19% decrease in the Lp(a) concentrations of obese patients. Only one study could be found concerning the effects of exercise on Lp(a) concentration and this was that of Hellsten and co-workers (1989). These authors reported a 22% decrease (from 16.6 mg.dl\(^{-1}\) to 12.9 mg.dl\(^{-1}\)) in the mean Lp(a) concentration of 16 men after eight days of cross-country skiing (seven to 16 miles per day). However, the validity of these findings is questionable due to the absence of a control group.
In the present study Lp(a) concentrations increased in both groups after 12 months (Table 6.3). The reason for these increases is not clear although they were possibly related to a storage effect i.e. the concentration of Lp(a) in the base-line samples, which were stored for a longer period, may have decreased resulting in the higher values found after 12 months. Little is known regarding the effects of storage on Lp(a) concentration and thus this suggestion remains speculative. The response of the two groups did not differ significantly, however, and there was no evidence that exercise had a beneficial influence on Lp(a) concentration.

6.2.5 Conclusion

A significant volume of training was performed by the walkers during this study which resulted in an improvement in the groups capacity for endurance exercise as discussed in Chapter Five. Despite the improvements in fitness, however, the walkers did not exhibit any beneficial changes in HDL-C concentration or in any of the other lipids, lipoproteins or apoproteins monitored during this study. Neither were there any beneficial changes in body weight or the percentage of body fat in the walking group. Thus, the amount of brisk walking performed during this study showed no potential for improving the serum lipid or lipoprotein concentrations of this group of previously sedentary, normolipidaemic, middle-aged men.
7 THE EFFECTS OF BRISK WALKING ON BLOOD PRESSURE, FIBRINOGEN AND PULMONARY FUNCTION

7.1 Introduction

As mentioned in the literature review hypertension is one of the major risk factors for CHD. Moreover, there is increasing evidence to suggest that elevations in plasma fibrinogen concentration and impairments in pulmonary function are also linked to the development of CHD. Furthermore, there is some evidence to suggest that exercise may be of benefit in lowering systolic and diastolic blood pressure and reducing plasma fibrinogen concentration. Exercise may also lead to improvements in pulmonary function. The purpose of this Chapter is to examine the effects of the brisk walking programme on each of these factors.

7.2 Methods

Resting arterial blood pressure was determined using a random zero sphygmomanometer (Section 3.6). Plasma fibrinogen concentration was measured using a coagulation assay (Sections 3.4 and 3.5; Appendix 14). A dry spirometer (Vitalograph Ltd.) was used to evaluate various lung function indices (Section 3.7). All of the above variables were measured at baseline and after three, six and 12 months.

The responses of the walkers and controls were compared using two way analysis of variance for repeated measures and relationships between variables were examined using the Pearson Product Moment correlation coefficient. Unless otherwise stated the number of subjects in each group is 42 and 23 for walkers and controls respectively.

7.3 Results

Systolic and diastolic blood pressure are displayed in Table 7.1. Plasma fibrinogen concentration is given in Table 7.2. Lung function indices including FVC, FEV₁ and FEV₁/FVC (%) are shown in Table 7.3. There were no significant interactions between the walking and control groups for any of these measures. There were significant changes through the year in plasma fibrinogen concentration and in FVC and FEV₁.
<table>
<thead>
<tr>
<th>Measure</th>
<th>Group</th>
<th>Baseline</th>
<th>3 months</th>
<th>6 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>W</td>
<td>115 ± 2</td>
<td>115 ± 2</td>
<td>115 ± 2</td>
<td>114 ± 2</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>122 ± 3</td>
<td>120 ± 3</td>
<td>123 ± 4</td>
<td>118 ± 3</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>W</td>
<td>79 ± 2</td>
<td>80 ± 2</td>
<td>79 ± 2</td>
<td>80 ± 2</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>81 ± 3</td>
<td>83 ± 2</td>
<td>84 ± 3</td>
<td>83 ± 2</td>
</tr>
</tbody>
</table>

Table 7.1 Resting arterial blood pressure (BP) for walkers and controls during the brisk walking study (mean ± SEM). No significant interactions.
<table>
<thead>
<tr>
<th>Group</th>
<th>Base line</th>
<th>3 months</th>
<th>6 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>W (n = 35)</td>
<td>238 ± 12</td>
<td>232 ± 11</td>
<td>240 ± 10</td>
<td>252 ± 11</td>
</tr>
<tr>
<td>C (n = 23)</td>
<td>251 ± 11</td>
<td>256 ± 16</td>
<td>259 ± 13</td>
<td>276 ± 15</td>
</tr>
</tbody>
</table>

Table 7.2 Plasma fibrinogen concentration (mg.dl⁻¹) for walkers and controls during the brisk walking study (mean ± SEM). No significant interaction.
<table>
<thead>
<tr>
<th>Measure</th>
<th>Group</th>
<th>Base line</th>
<th>3 months</th>
<th>6 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC (Litres)</td>
<td>W</td>
<td>5.0 ± 0.1</td>
<td>4.9 ± 0.1</td>
<td>4.8 ± 0.1</td>
<td>4.8 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>4.9 ± 0.1</td>
<td>4.8 ± 0.1</td>
<td>4.7 ± 0.2</td>
<td>4.8 ± 0.1</td>
</tr>
<tr>
<td>FEV₁ (Litres)</td>
<td>W</td>
<td>3.9 ± 0.1</td>
<td>3.9 ± 0.1</td>
<td>3.7 ± 0.1</td>
<td>3.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>3.6 ± 0.1</td>
<td>3.6 ± 0.1</td>
<td>3.4 ± 0.1</td>
<td>3.6 ± 0.1</td>
</tr>
<tr>
<td>FEV₁/FVC (%)</td>
<td>W</td>
<td>77.8 ± 0.9</td>
<td>77.7 ± 0.8</td>
<td>77.7 ± 0.8</td>
<td>77.4 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>74.2 ± 1.0</td>
<td>74.1 ± 1.0</td>
<td>73.8 ± 1.0</td>
<td>74.4 ± 1.1</td>
</tr>
</tbody>
</table>

Table 7.3 Lung function measures for walkers and controls during the brisk walking study (mean ± SEM). No significant interactions.
7.4 Discussion

Throughout the study mean values (Table 7.1) for resting arterial blood pressure in both the walking and control groups were close to values which would be considered normal (i.e. 120/80 mm Hg). An examination of the individual data revealed that 11 of the subjects (six walkers and five controls) were mild hypertensives at the start of the study according to the criteria of Tipton (1991) i.e. systolic and/or diastolic blood pressure >140/90 mm Hg. Of these subjects, nine had elevated diastolic blood pressure, one had elevated systolic blood pressure and one had elevations in both diastolic and systolic blood pressure. There were significant but low correlations between predicted VO$_2$max and both systolic and diastolic blood pressure at base line when all 65 subjects were included in the analysis ($r = -0.27$ and $-0.30$ respectively). This would indicate that higher VO$_2$max values were to some extent associated with lower resting systolic and diastolic blood pressure.

The lack of change in blood pressure for the walking group as a whole is not unexpected since base-line blood pressure was not elevated. Other studies examining the effects of exercise on blood pressure in normotensive subjects have also failed to show significant changes in blood pressure with training (Gilders et al. 1989; Verity et al. 1990). Moreover, Tipton (1984) in his extensive review concerning the effects of exercise on blood pressure concluded that reductions were only likely to occur in normotensive subjects whose initial values were above 125/85 mm Hg.

To pursue the premise of Tipton (1984) more thoroughly changes in blood pressure were examined in two subgroups of walkers. One of these subgroups contained eight individuals whose systolic blood pressure was found to be above 125 mm Hg at base line. The other group consisted of 14 subjects whose diastolic blood pressure was initially above 85 mm Hg. The results are displayed in Table 7.4 below. From this Table it can be seen that there were mean reductions of 3 mm Hg in both systolic and diastolic blood pressure after 12 months. These are modest reductions especially when considering the fact that the control group experienced a 4 mm Hg reduction in systolic blood pressure after 12 months (Table 7.1). However, an examination of the range of values reveals that although the top end of the range remains relatively stable during the study, the bottom end of the range is reduced.
substantially in each group. Thus, there is some evidence that exercise had a beneficial influence here.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Base line</th>
<th>3 months</th>
<th>6 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP</td>
<td>8</td>
<td>134 ± 3</td>
<td>131 ± 4</td>
<td>130 ± 6</td>
<td>131 ± 5</td>
</tr>
<tr>
<td>&gt;125 mm Hg</td>
<td></td>
<td>126 - 148</td>
<td>116 - 156</td>
<td>100 - 150</td>
<td>112 - 146</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>14</td>
<td>91 ± 2</td>
<td>88 ± 3</td>
<td>91 ± 2</td>
<td>88 ± 3</td>
</tr>
<tr>
<td>&gt;85 mm Hg</td>
<td></td>
<td>86 - 102</td>
<td>68 - 104</td>
<td>76 - 104</td>
<td>58 - 102</td>
</tr>
</tbody>
</table>

Table 7.4 Systolic and diastolic blood pressure (BP) during the brisk walking study for walkers whose base-line values were above 125 mm Hg and 85 mm Hg respectively. For each variable the top row gives the mean ± SEM and the bottom row gives the range.

For the walking group as a whole there were no significant correlations between the average minutes or miles of brisk walking completed each day and the changes in systolic or diastolic blood pressure after three, six or 12 months (i.e. three month values minus base-line values etc.). Similarly training intensity (%HRmax) did not correlate significantly with changes in blood pressure. When the same relationships were examined for the subgroups of subjects in Table 7.4, however, some interesting findings emerged. For the eight walkers whose base-line systolic blood pressure was above 125 mm Hg, changes in systolic blood pressure after six and 12 months correlated significantly with the average miles of brisk walking completed per day ($r = -0.78$ and $-0.87$ respectively). Furthermore, average minutes of brisk walking completed each day had an even stronger relationship with changes in systolic blood pressure after 12 months ($r = -0.93$) (Figure 7.1). Similarly there were significant correlations between the average miles of brisk walking completed per day and changes in diastolic blood pressure after three and six months in the 14 subjects whose base-line diastolic blood pressure was above 85 mm Hg ($r = -0.60$ and $-0.58$ respectively). Thus, within these subgroups, the subjects who walked the most miles each day were likely to have the greatest reductions in systolic and diastolic blood pressure during the study.
Figure 7.1 The relationship between the average minutes of brisk walking per day and 12 month changes in systolic blood pressure (SBP) in eight walkers whose base-line values were above 125 mm Hg.

There were no significant correlations between training intensity and changes in blood pressure in the subgroups of subjects discussed above. This supports the findings of Roman and colleagues (1981) that reductions in arterial blood pressure are not necessarily dependent on training intensity.

In summary then, brisk walking did not influence resting arterial blood pressure in this group of normotensive middle-aged men. There were some indications, however, that exercise had a beneficial influence on blood pressure if base-line values were elevated above either 125 mm Hg in systole or 85 mm Hg in diastole. In these cases there was evidence of a dose response relationship with the greatest reductions in blood pressure occurring in those subjects who walked the most miles.

Plasma fibrinogen concentration was determined in the present study due to the findings from prospective studies such as the Northwick Park Heart Study (Meade et al. 1986) which have shown that high concentrations of plasma fibrinogen predispose individuals to an increased risk of CHD. The mechanisms responsible for the link between plasma fibrinogen concentration
and CHD are not fully understood but high fibrinogen concentrations may promote the development of atherosclerotic plaques in addition to increasing the likelihood of clot formation in coronary vessels.

The mean plasma fibrinogen values of the walkers and controls in the brisk walking study (Table 7.2) are lower than those obtained for healthy individuals in many of the prospective studies examining plasma fibrinogen and CHD (Table 7.5). This probably reflects the fact that the subjects in the present study were non or occasional smokers, since smoking is known to influence plasma fibrinogen concentration. For example, in the Northwick Park Heart Study (Meade et al. 1986) plasma fibrinogen concentrations were 30 mg.dl⁻¹ lower in non-smokers compared with smokers. The plasma fibrinogen values in the present study are similar to those of the non-smokers in the Northwick Park Heart Study (Meade et al. 1986) i.e. 275 mg.dl⁻¹.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Age at entry (yrs)</th>
<th>Developed CHD</th>
<th>Healthy/free of CHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meade et al. 1986</td>
<td>40 - 64</td>
<td>315 mg.dl⁻¹ (n = 109)</td>
<td>290 mg.dl⁻¹ (n = 1280)</td>
</tr>
<tr>
<td>Stone and Thorpe, 1985</td>
<td>40 - 69</td>
<td>392 mg.dl⁻¹ (n = 40)</td>
<td>300 mg.dl⁻¹ (n = 209)</td>
</tr>
<tr>
<td>Wilhelmsen et al. 1984</td>
<td>54</td>
<td>356 mg.dl⁻¹ (n = 92)</td>
<td>330 mg.dl⁻¹ (n = 608)</td>
</tr>
<tr>
<td>Yarnell et al. 1991</td>
<td>45 - 59</td>
<td>409 mg.dl⁻¹ (n = 233)</td>
<td>366 mg.dl⁻¹ (n = 4408)</td>
</tr>
</tbody>
</table>

Table 7.5 Plasma fibrinogen concentration at base line for healthy individuals and for those who subsequently developed coronary heart disease in the Northwick Park Heart Study (Meade et al. 1986), the Leigh Study (Stone and Thorpe, 1985), the Goteborg Study (Wilhelmsen et al. 1984) and the Caerphilly and Speedwell collaborative heart disease Studies (Yarnell et al. 1991).
Plasma fibrinogen concentration did not significantly differ between walkers and controls during the study although there was a significant increase in plasma fibrinogen in both groups as the study progressed. The reason for this increase is unknown although it may be due to the effects of storage. No other longitudinal studies examining the effects of exercise on plasma fibrinogen concentration could be found but the results from the present study do not concur with previous reports that endurance trained individuals have lower fibrinogen levels than sedentary controls (Ernst, 1990). As with the changes in blood pressure discussed above the lack of an exercise effect may be partly explained by the fact that fibrinogen values were relatively low at base line. It is also possible that the exercise intensity was too low to have an effect on plasma fibrinogen. Connelly and colleagues (1992) have recently shown, for example, that strenuous but not mild exercise is associated with decreased plasma fibrinogen concentrations.

The mean values for the lung function parameters of the subjects in the present study (Table 7.3) are similar to previously reported values for lung function in healthy males aged 52 years and standing 1.78 metres tall i.e. FVC: 4.6 litres; FEV₁: 3.7 litres; FEV₁/FVC (%): 77.9 % (European Community for Coal and Steel Standardised Lung Function Testing, 1983). The reason for the decrease in FVC (4% in the walkers and 2% in the controls) and FEV₁ (5% in the walkers) at the end of the study is unknown. According to Astrand and Rodahl (1986) FVC and FEV₁ decrease by around 0.4% per year in healthy males aged between 20 and 70 years old. This may account for some, though not all, of the decrease seen in the present study.

It has previously been reported by Newman and colleagues (1961) that physically active people have enhanced pulmonary function values. More recently a positive association between FEV₁ and physical activity was found in the British Regional Heart Study (Shaper and Wannamethee, 1991). In this study a physical activity index was calculated for each individual using questionnaires. Individuals were then categorised into groups according to their physical activity score. The results revealed a positive and graded response between physical activity and FEV₁ throughout the groups. The findings in the present study do not conform with the above studies but are consistent with previous reports that marathon runners (Kaufmann et al. 1974) and competitive swimmers (Ness et al. 1974) have similar values for pulmonary function to those of their sedentary peers.
When the 65 subjects from the present study were examined as a single group some significant relationships between base-line measures of pulmonary function and other CHD risk factors emerged. Height adjusted FEV₁ (FEV₁/Ht²) correlated positively with VO₂max (r = 0.26) and HDL-C (r = 0.34) and negatively with triglyceride concentration (r = -0.33) and body fat percentage (r = -0.45). These findings are remarkably similar to those of Higgins and colleagues (1991) who observed that height adjusted FEV₁ was correlated positively with a history of strenuous physical activity, duration of treadmill exercise and HDL-C, and negatively with serum triglyceride and skinfold thicknesses in the CARDIA participants. Forced vital capacity and FEV₁ were also significantly and negatively correlated with systolic blood pressure at base line (r = -0.32 and -0.27 respectively) providing some evidence to support previous reports of a relationship between impaired lung function and arterial hypertension (Selby et al. 1990; Sparrow et al. 1988). Taken together these findings are consistent with the hypothesis that measures of pulmonary function are related to the incidence of heart disease through their association with other CHD risk factors.

7.5 Conclusion

Brisk walking had no influence on resting arterial blood pressure, plasma fibrinogen concentration or pulmonary function in this group of previously sedentary, asymptomatic, middle-aged men.
This study sought to examine the potential of brisk walking to influence endurance fitness and to modify some of the established risk factors for CHD in a group of previously sedentary, middle-aged men. These men were asymptomatic for CHD as defined by many of the commonly accepted CHD risk factors. For example, as a group the men were not obese, hyperlipidaemic or hypertensive. Moreover, the group was composed of only non or occasional smokers. Nevertheless, although the individual risk of CHD was low, a large number of deaths occur in these sort of men in the U.K. each year.

In addition to the commonly accepted risk factors for CHD such as total cholesterol, HDL-C and blood pressure, other variables which have only more recently been added to the CHD risk factor list, including plasma fibrinogen, Lp(a) concentration and measures of pulmonary function, were examined. The main findings of the study were that brisk walking had a significant influence on the endurance fitness of the subjects but had no measurable affect on any of the CHD risk factors examined.

The physiological and metabolic changes indicative of enhanced endurance fitness were examined in Chapter Five. The most pronounced change to occur in the walkers was the marked reduction in blood lactate concentration during standardised submaximal exercise. This reduction averaged around 35% and confirmed initial expectations that endurance training invariably results in metabolic adaptations i.e. improvements in the oxidative capacity of skeletal muscle, in the absence of profound changes in VO2max.

The specific adaptations which would lead to a reduction in blood lactate concentration during standardised submaximal exercise were discussed in Chapter Two. Possible changes would include an increase in the size and number of mitochondria within skeletal muscle and an increase in mitochondrial enzyme activity. These adaptations in turn would be likely to enhance fat metabolism during exercise (of which there was some evidence in the reduced R values of the walkers) and perhaps also to improve control over glycogenolysis as suggested by Matoba and Gollnick (1984) and Holloszy and Coyle (1984). As a consequence of these changes a reduced muscle glycogen utilisation would be expected at a given submaximal exercise
intensity which would result in a sparing of muscle glycogen and therefore an improved capacity for endurance exercise.

It is also possible that the reductions in blood lactate concentration during submaximal exercise were related to increases in the capillary density of skeletal muscle. Ingjer (1979), for example, found a significant increase in the average number of capillaries per muscle fibre in young females who trained by cross-country running over a 24 week period. Astrand and Rodahl (1986) state that an increase in capillary density will reduce the distance between the blood and the cell interior and therefore enhance the exchange of gases substrates and metabolites. This would allow for a greater aerobic contribution to energy provision at a given exercise intensity thus reducing the flux through the glycolytic pathway. It is not known whether exercise such as brisk walking can increase muscle capillarisation but bearing in mind the above evidence it is certainly a possibility.

There was some indication also of cardiovascular adaptation within the walking group. Although VO$_2$max was not increased there was a significant reduction in heart rate during standardised, submaximal exercise. As mentioned in Chapter Five, it is possible that the lack of change in VO$_2$max was due to inaccuracies in prediction caused by alterations in the oxygen uptake/heart rate relationship. An increase in VO$_2$max would not necessarily be expected with low intensity exercise but previous studies have shown that elevations in VO$_2$max are possible after a period of brisk walking training (Jette et al. 1988; Seals et al. 1984a). The reductions in heart rate during submaximal exercise may have been due partly to a decreased arousal response to treadmill walking but it is possible also that there was a small increase in both maximal and submaximal stroke volume. If this was the case then a lower heart rate would be required to attain a given cardiac output.

The lack of change in any of the established risk factors for CHD was disappointing and in the case of HDL-C perhaps surprising in the light of previous studies which have shown increases in HDL-C after various periods of endurance training. In the present study there was no indication that exercise had a beneficial influence on HDL-C. It is possible that the lack of change in HDL-C could have been related to many factors acting separately or in conjunction with one another. For example, the initially high HDL-C concentrations of the subjects and the low training intensity used relative to
many other studies may together explain the lack of change in HDL-C concentration. Coupled with the above factors it may be that the duration of the exercise programme was too short to provoke detectable changes in HDL-C. Williams and colleagues (1982) observed in their one year training study, for example, that endurance fitness improved sooner and at lower exercise levels than required for HDL-C concentration changes. However, in the present study there was no increase in HDL-C in a subgroup of 14 walkers who continued to train for a second year (Sara Herd, personal communication).

One of the primary mechanisms which has been forwarded to explain how exercise might increase HDL-C concentration involves the enzyme lipoprotein lipase (see Chapter Two, Section 2.5.6). Lipoprotein lipase facilitates the removal of triglyceride from chylomicrons and VLDL. In this process cholesterol, protein and phospholipid remnants are transferred to ‘nascent’ HDL particles thus augmenting HDL-C production. It has been shown that endurance training can lead to an increase in lipoprotein lipase activity (Peltonen et al. 1981; Thompson et al. 1988). Moreover, in a study by Kiens and Lithell (1989), eight weeks of dynamic exercise training resulted in an increased capillarisation of skeletal muscle as well as a 70% elevation in muscle lipoprotein lipase activity and an 8% increase in HDL-C concentration.

Bearing in mind the above findings it is again surprising that the brisk walking programme did not result in an increase in HDL-C concentration since there was convincing evidence to suggest that the oxidative capacity of skeletal muscle was enhanced in the walking group. This enhanced capacity was possibly related to an increased activity of the enzymes involved in fat metabolism in addition to an improved supply of substrate, and thus triglyceride, to the muscle for use as an energy source. This in turn would suggest a greater triglyceride dissolution and according to the above evidence an increased formation of HDL-C. However, this did not appear to be the case.

It is possible that the lack of change in HDL-C in the present study may be explained on the basis of energy expenditure. According to the results of the seven day weighed food inventories completed by the subjects after six and 12 months there was no increase in energy intake in the walking group during the study. Moreover, body fat percentage was not significantly reduced in the walking group either. Therefore, it is feasible that the walkers may have compensated for the increased energy which they expended in walking by
reducing the amount of energy they expended in other activities. If this was the case, overall energy expenditure would have been unchanged and thus lipoprotein lipase activity and triglyceride hydrolysis may have remained constant.

The results of this study are in sharp contrast to those of the women's study previously performed in this laboratory in which a one year programme of brisk walking was effective in increasing HDL-C concentration by 27% (Hardman et al. 1989a; Hardman and Hudson, 1991). This difference is not easy to explain. The males in the present study did more walking than the females in the previous study (28 minutes per day or approximately 14 miles per week for the males versus 22 minutes per day or 11 miles per week for the females). Average brisk walking speed was also faster for the males (males: 1.90 m.s⁻¹ (4.3 miles.hour⁻¹) at base line; 1.98 m.s⁻¹ (4.4 miles.hour⁻¹) after 12 months; females: 1.72 m.s⁻¹ (3.8 miles.hour⁻¹) at base line; 1.87 m.s⁻¹ (4.2 miles.hour⁻¹) after 12 months). However, the average relative exercise intensity was similar in both studies at around 60% of predicted VO₂max. In both studies also there were indications of improved endurance fitness i.e. reductions in heart rate and blood lactate concentration during submaximal exercise, but body fatness did not change in either study.

One explanation for the disparate findings between the two studies is that they are due to a sex difference. It may be, for example, that in women the interaction of exercise with the sex hormone estrogen causes a more profound effect on HDL-C than that found in men. However, there is no clear evidence to support this suggestion. Previous longitudinal studies examining the effects of exercise on HDL-C in both men and women with have yielded conflicting results. For example, Brownell and colleagues (1982) found an increase in HDL-C in men but not in women while Hill and co-workers (1989) found the exact opposite i.e. an increase in HDL-C in women but not in men and Farrell and Barboriak (1980) observed an increase in HDL-C in both men and women. If there is a consistent sex related difference in the HDL-C response to training it is not obvious from these studies. Moreover, although one previous review of the literature concluded that there may be a sex difference this was in the favour of men i.e. women were thought less likely than men to experience changes in HDL-C with exercise training (Goldberg and Elliot, 1987). However, Wood and colleagues (1984), in their review on HDL-C and
exercise, concluded that there was no real evidence to support the suggestion that women are less likely than men to increase their HDL-C.

It should be noted that the increase in HDL-C in the women's study, while being consistent with the differences found in cross-sectional studies, is unusually large compared with many longitudinal studies examining both men and women. Haskell (1986) states that the differences in HDL-C between trained and untrained subjects in observational studies are usually between 20 and 35% while increases in HDL-C with training are usually in the order of 5 to 16%. Thus, it could be that the change in HDL-C in the women's study was related to other factors as well as exercise. Increases in alcohol or fat consumption may cause increases in HDL-C, for example. However, there was no indication from seven day weighed food intake records that any qualitative changes occurred in the women's diets. Moreover, even if this had been the case the change in HDL-C was too great to be explained by modest dietary change alone (Hardman and Hudson, 1991). Oral contraceptives and hormone replacement therapy are also known to influence lipoproteins and therefore it is possible that these could have elicited changes in HDL-C in the women. Even low doses of hormone replacement therapy can increase HDL-C dramatically when estrogens only are used (Miller and La Rosa, 1991). However, only two of the 28 walkers in the women's study were on hormone replacement therapy (Jones et al. 1991). Moreover, although two other walkers were taking oral contraceptives these usually decrease HDL-C or it remains unchanged (Miller and La Rosa, 1991).

Aside from sex another obvious difference between the subjects which may explain the incompatible findings of the two studies is the baseline values for HDL-C. Baseline HDL-C concentration was lower in the females than the males (1.2 mmol.l⁻¹ for women versus 1.4 mmol.l⁻¹ for men). This is surprising since women usually have higher HDL-C concentrations than men (Goldberg and Elliot, 1987). Although the precipitation procedures used in the two studies were dissimilar (phosphotungstic acid and magnesium chloride versus manganese chloride and sodium heparin in the women's and men's studies respectively) the difference between these methods (a maximum of 0.1 mmol.l⁻¹, Vallance, personal communication) is not large enough to explain the lower values of the women. Accepting the results at face value then it is possible that there was a greater potential for an exercise induced increase in
HDL-C concentration in the women because their initial HDL-C concentration was lower than that of the men.

Finally, physical fitness levels in the women appear to have been poor when they began their study. The mean value for predicted VO\(_2\)max at base line, for example, was 26.7 ml.kg\(^{-1}\)min\(^{-1}\) in the women. This is low considering that the average age of the women was 45 years and that the average predicted VO\(_2\)max value obtained for women aged 45 to 54 in the National Fitness Survey was 31.9 ml.kg\(^{-1}\)min\(^{-1}\) (The Sports Council and the Health Education Authority, 1992). It is feasible, therefore, that the combination of low HDL-C and poor absolute fitness in the women meant that they were especially susceptible to increases in HDL-C with training. It is true that the average VO\(_2\)max of the men was also low compared with men of similar age in the National Fitness Survey, but poor fitness did not co-exist with low HDL-C concentration in the men’s study as it did in the women’s study. Despite all this conjecture and in the absence of more conclusive evidence, however, the difference between the two studies remains unresolved.

Returning to the present study, the fact that the brisk walking programme did not influence any of the other CHD risk factors examined in the men was less surprising than its ineffectiveness in influencing HDL-C because the effects of exercise on these other factors are more contentious than are the effects of exercise on HDL-C. Moreover, the walking group as a whole did not exhibit undesirable levels of many of these risk factors to begin with. Although the mean total cholesterol concentration of the walking group was above ideal, for example, it was still below the national average for men of similar age. Moreover, mean blood pressure, plasma fibrinogen and lung function values for the walking group were not undesirable at the outset of the study and thus their potential for change may have been limited.

The characteristics of the subject group may partly explain some of the findings in the present study then. These characteristics were dictated to a great extent by the subject selection criteria, which excluded those already at high risk of CHD, and perhaps also by the randomisation procedures used. In short the subject group represented a sample of convenience comprising only those who were willing to take part. Moreover, Kraemer (1981) has pointed out that the process of randomisation in clinical trials tends to exacerbate recruitment problems and dropout rates since subjects who are allocated into
placebo groups often feel that they have little to gain from their participation. Thus, the possibility of being assigned to the control group may have dissuaded many men who felt that they were at risk i.e. had the potential for lifestyle change, from participating in the present investigation. As a result, the subjects studied may have been healthier than average and as noted by Kraemer (1981), "patients who are relatively most healthy tend to be very unresponsive to treatment".

What are the implications of these findings? Do they suggest that brisk walking is an inappropriate means of reducing CHD risk? Bearing in mind the epidemiological evidence the answer to this question must be no. As mentioned in Chapter Two, many epidemiological studies have demonstrated an inverse link between physical activity and mortality from CHD. On the basis of these studies Powell and colleagues (1987) have calculated that sedentary individuals have an approximately twofold greater chance of suffering from CHD compared with their physically active counterparts. Moreover, some epidemiological studies have specifically examined walking exercise and again the results have been positive. In the British Regional Heart Study (Shaper and Wannamethee, 1991), for example, men who walked 41 to 60 minutes per day showed a decreased risk of heart attack compared with less active men. Paffenbarger and colleagues (1986) also examined walking exercise and found that all-cause mortality was 21% lower in men who walked nine miles or more per week compared with those who walked below three miles per week. Moreover, in the most recent study of Morris and colleagues (1990) men who "... rated the pace of their regular walking as fast (>4 mph) experienced less than half the non-fatal and fatal CHD of other men".

In the present study, 86% of the walkers trained at a pace which was faster than 4 mph and 83% of the walkers covered nine or more miles per week. According to the above epidemiological evidence, this would confer a reduced risk of CHD provided the walking was continued on a regular basis. Moreover, although brisk walking did not influence any of the established CHD risk factors examined in the present study it did have a significant influence on endurance fitness. This would support the contention that at least part of the association between exercise and heart disease may be attributable to a direct and independent influence of exercise itself. Perhaps, as Morris and colleagues (1990) suggest, endurance fitness protects against CHD. This suggestion is substantiated by the results of many epidemiological studies
which have shown that exercise is independently related to the incidence of CHD even after the relationship between exercise and other risk factors has been adjusted for.

The mechanisms which may explain the independent relationship between exercise and CHD are uncertain but many possibilities exist. For example, exercise training enhances coronary collateral development in dogs (Neill and Oxendine, 1979) and can retard atherosclerosis and enlarge the coronary arteries of monkeys (Kramsch et al. 1981). Moreover, an autopsy study on the marathon runner Clarence DeMar revealed that his coronary arteries were two to three times the normal diameter (Currens and White, 1961). Additionally, exercise may contribute to the "general health" of the heart by reducing resting heart rate, increasing stroke volume and therefore improving the efficiency of heart muscle (Astrand, 1988). Thus, it is biologically plausible that exercise training may reduce CHD risk by increasing myocardial oxygen supply or by reducing myocardial oxygen demand.

Epidemiological studies such as those of Morris and colleagues (1990) and Paffenbarger and co-workers (1978) have shown that only those individuals who maintain high physical activity levels are at reduced risk of CHD, while a previous history of physical activity alone is not protective. On the basis of these results it may be that exercise protects from the acute phases of CHD rather than influencing its development. It has been shown, for example, that exercise training enhances the electrical stability of the heart (Hollmann, 1988) and even modest physical activity associated with a "... minimal improvement in physical fitness can reduce vulnerability of the myocardium for fatal ventricular dysrhythmias" (Leon, 1988).

Another possibility relates to thrombosis. Blair and colleagues (1992) state that "total occlusion of a coronary artery as a result of thrombus formation at the site of an atherosclerotic stenosis is believed to be the final precipitating event in more than 90% of acute myocardial infarctions". Exercise training has been shown to reduce blood and plasma viscosity (Ernst, 1985; Ernst, 1987) and platelet adhesiveness and aggregability (Eichner, 1986; Rauramaa et al. 1986). Furthermore, acute exercise increases fibrinolytic activity (El-Sayed, 1990). Thus, exercise training may reduce the likelihood of thrombus formation and/or lead to an improvement in the body's ability to dissolve thrombi.
Tunstall-Pedoe and Smith (1990) have emphasised that CHD risk is multifactorial and that the "...management of risk factors in individuals should involve... a flexible and multi-dimensional individual regimen, comprising all modifiable factors". In view of this and the above evidence, brisk walking ought to be considered as part of an intervention package by which CHD risk can be modified. There is much to recommend brisk walking in this role. Apart from the possibility that it may directly influence CHD risk, Tunstall-Pedoe (1990) has emphasised that exercise also "...makes people think about their bodies, what they can do with them and how they should eat, (and therefore) performs a useful promotional function". Moreover, brisk walking is an acceptable form of exercise. This was demonstrated clearly in the present study in which 42 of the original 48 walkers (88.5%) continued to exercise regularly throughout. This represents only a 12.5% attrition rate which is substantially lower than the attrition rates typifying many exercise programmes i.e. 50% or more according to Martin and Dubbert (1982). The incidence of injuries in the present study was also very low which was especially encouraging considering that the men were previously sedentary. This supports the evidence from other studies that orthopaedic injuries occur more frequently during running programmes than during walking programmes (Seals et al. 1984a).

Astrand (1988) argues that "from many points of view, it is a risk factor to quickly change from the life style of a huntergatherer to one of an urbanised high-technologist". The epidemiological evidence supports this viewpoint and demonstrates that individuals who perform moderate amounts of brisk walking on a regular basis are at a decreased risk of suffering from CHD in comparison with individuals who are less active. It remains to be shown exactly how brisk walking lowers an individuals risk of contracting CHD. However, brisk walking does not appear to be influencing CHD risk through any of the mechanisms which this study has examined markers for. Therefore, the results of this study strengthen the hypothesis that endurance fitness, in its own right, protects against CHD.
REFERENCES


APPENDICES
Appendix 1: Information supplied to the subjects and statement of Informed consent

BRITISH HEART FOUNDATION PROJECT - 1989

Purpose of the study:

The purpose of the study is to examine the influence of regular brisk walking on i) physical fitness, ii) the amount and distribution of body fat, iii) the concentration in blood of cholesterol and other markers of coronary heart disease risk and iv) psychological well being.

Criteria for acceptance into the study:

1. Aged 42-59 on entry into the study.
2. Neither currently on a programme of regular exercise nor employed in a strenuous job.
4. Not at high risk of coronary heart disease (CHD), i.e. non-smoker or occasional smoker, blood pressure and blood cholesterol below the high range.
5. Willing to accept random allocation to either the Walking Group or to the Control Group, with a 2 : 1 chance of being in the Walking Group. A programme of walking and monitoring will be conducted for the Control Group after the present study is complete.

Explanation of the procedures:

A) Four exercise tests will be undertaken, three of these in the laboratory - after a practice session to become familiar with the apparatus.

i) Test one involves walking uphill on a motorised treadmill at a fixed speed for 16 minutes. The gradient will be increased at the end of each four minute period. Heart rate will be recorded throughout from three chest electrodes and expired air will be collected at intervals using a mouthpiece and respiratory valve.

ii) The test described above will be repeated but using treadmill gradients selected to represent 50%, 60%, 70% and 80% of each man's estimated maximal capacity. Five very small (20 µl) samples of blood will be obtained by pricking the thumb, before exercise and at the end of each four minute period of walking.
iii) On another occasion, subjects will walk for 20 minutes on the motorised treadmill at a gradient selected to be equivalent to 70% of their own estimated maximum capacity. Heart rate and expired air will be collected as described above and thumbprick blood samples will be taken before and on three occasions during the test. In addition, a 10 ml blood sample will be taken from a vein in the arm before and after the test. Subjects will also exhale maximally into an instrument which measures lung function before and after exercise.

iv) Finally, each subject will walk one mile outside, at a brisk pace. During the walk heart rate will be recorded from two chest electrodes using short range telemetry.

B) The amount of fat under the skin will be determined using calipers at ten different sites and a number of body girths will be measured. Total body fat will be determined from body density derived by underwater weighing. This is done in a tank of warm water (36 °C) and involves breathing out maximally through a snorkel whilst submerged. The volume of air left in the lungs, after breathing out as far as possible, is determined by taking three deep breaths from a bag of 100% oxygen fitted to the snorkel.

C) Two, 20 ml samples of blood will be taken from a vein in the arm for the analysis of serum cholesterol and other markers for CHD. This needs to be done in the morning after a 12 hour fast. Haemoglobin concentration and the proportion of red cells in blood will also be measured in these blood samples.

D) Resting blood pressure will be measured.

E) All food and drink consumed over a period of one week will be weighed and recorded so that average daily nutrient and energy intake can be determined.

F) Several questionnaires will be completed. These can all be done at home. They ask questions about i) your current physical activity level, ii) previous and past smoking habits and, iii) how you feel about yourself (self-esteem).

Walking:

Those allocated to the Walking Group will follow a brisk walking programme. This will be individually prescribed on a fortnightly basis and will increase to an average of about three miles a day by the end of six months and thereafter. The first session will be on the University site so that each man's self-selected brisk pace can be determined.

The Control Group will be asked to maintain their habitual lifestyle until this trial is complete. After one year the subjects in this group will embark on the brisk walking programme described above.
Repeated measurements:

The measurements described above, with the exception of the underwater weighing, will be repeated after three months of regular walking and again after six months. After one year all measurements, including the underwater weighing, will be repeated.

Risks and discomforts:

Although all exercise testing will be at a submaximal level, the possibility exists that, very occasionally, certain changes may occur during the test. They include abnormal blood pressure, fainting, disorder of the heart beat and, in very rare instances, heart attack. The preliminary screening undertaken before the study is designed to minimise such risks. Sampling of venous blood may cause minor bruising or haematoma (a small accumulation of blood under the skin). All experiments will be conducted by trained and experienced staff and closely monitored. You should feel free to ask for more information or explanation at any time.

Benefits:

The trial is being conducted because it is not known whether regular, brisk walking can influence fitness and/or health in men. There can therefore be no guarantee that you personally will benefit from taking part. We do, however, have every expectation that you will enjoy contributing to a worthwhile study. The very least you will gain is a greater understanding of the body's responses to exercise.

If you need more information please contact David Stensel (4326), Katherine Brooke-Wavell (3005) or any other member of the BHF Research Group.

Dr Adrianne Hardman (3265)
Dr Peter Jones (3003)
Dr Nick Norgan (3009)
Statement of informed consent.

I understand that I will be asked to undertake the series of tests described above. All procedures have been described and explained to me and I am aware that I may withdraw from the study at any time without the need for explanation.

I agree to take part in the British Heart Foundation walking study.

Signed ............................................................... .

Date .................................. .

Witnessed by ......................................................... .
Appendix 2: The calculation of oxygen uptake and carbon dioxide production

\[ \text{VE}_{\text{STPD}} = \text{VE}_{\text{ATPS}} \times \frac{(\text{BP} - \text{SWVP})}{760} \times \frac{273}{273 + t} \]

Where:

- \( \text{BP} \) = barometric pressure in mm Hg.
- \( \text{SWVP} \) = saturated water vapour pressure (mm Hg) at ambient temperature.
- \( t \) = ambient temperature (degrees Celsius).
- \( \text{ATPS} \) = ambient temperature and pressure, saturated with water vapour.

\( \text{SWVP}_t \) was calculated as follows: \((1.001 \times t) - 4.19\)

\( \text{VO}_2 = \text{Vol. O}_2 \text{ inspired} - \text{Vol. O}_2 \text{ expired}. \)

\[ \text{Vol. O}_2 \text{ inspired} = \text{VI} \times \frac{\text{FiO}_2\%}{100} \]

\[ \text{Vol. O}_2 \text{ expired} = \text{VE} \times \frac{\text{FeO}_2\%}{100} \]

Similarly \( \text{VCO}_2 = \text{Vol. CO}_2 \text{ expired} - \text{Vol. CO}_2 \text{ inspired}. \)

\[ \text{VCO}_2 = \left(\frac{\text{VE} \times \text{FeCO}_2\%}{100}\right) - \left(\frac{\text{VI} \times \text{FiCO}_2\%}{100}\right) \]

It is assumed that \( \text{FiO}_2\% \) is 20.93% and \( \text{FiCO}_2\% \) is 0.03% as the composition of inspired air is very stable.

\( \text{FeO}_2\% \), \( \text{FeCO}_2\% \) and \( \text{VE} \) are measured in the expired air sample. \( \text{VI} \) is derived from the Haldane transformation.
The Haldane transformation uses the concentration of nitrogen (assumed to be metabolically inert) in the inspired and expired air to derive the volume of air inspired from direct measurements of the volume expired.

i.e. As concentration = \[\frac{\text{mass}}{\text{volume}}\]

\[\text{mass } N_2 \text{ inspired } = F_{I}N_2\% \text{ and } \frac{\text{VI}}{	ext{VE}}\]

\[\text{mass } N_2 \text{ expired } = F_{E}N_2\% \text{ and } \frac{\text{VE}}{\text{FI}N_2\%}\]

But mass of $N_2$ inspired = mass of $N_2$ expired

therefore \[F_{I}N_2\% \times \text{ VI } = F_{E}N_2\% \times \text{ VE}\]

and \[\text{ VI } = \frac{F_{E}N_2\%}{F_{I}N_2\%} \times \text{ VE}\]

VI can therefore be derived if VE is measured and $F_{I}N_2\%$ and $F_{E}N_2\%$ are known.

$F_{I}N_2\%$ and $F_{E}N_2\%$ are found by subtracting the concentration of carbon dioxide and oxygen from 100%.

Finally, the respiratory exchange ratio (R) is calculated as: \[\frac{VCO_2}{VO_2}\]
Appendix 3: Treadmill calibration

Treadmill calibration took place every three months during the brisk walking study. Calibration was performed by measuring the length of the treadmill belt and the time taken for it to complete 50 revolutions at the speeds used in the tests (i.e. 1.34 m.s\(^{-1}\) and 1.56 m.s\(^{-1}\)). By multiplying the length of the belt by 50 this gave the total distance (in metres) the belt would have travelled. This distance was then divided by the number of seconds taken to complete it to determine the actual speed at which the belt was travelling. The Table below gives the actual speeds obtained during the calibrations where the dial speeds were set at 1.34 m.s\(^{-1}\) and 1.56 m.s\(^{-1}\). At the bottom of the Table the mean, standard deviation (SD) and coefficient of variation (CV) for each speed are shown.

<table>
<thead>
<tr>
<th>Calibration</th>
<th>Dial speed = 1.34 m.s(^{-1})</th>
<th>Dial speed = 1.56 m.s(^{-1})</th>
</tr>
</thead>
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<tr>
<td>Base line</td>
<td>1.38</td>
<td>1.64</td>
</tr>
<tr>
<td>3 months</td>
<td>1.33</td>
<td>1.59</td>
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<tr>
<td>6 months</td>
<td>1.38</td>
<td>1.59</td>
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<tr>
<td>9 months</td>
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</tr>
<tr>
<td>SD</td>
<td>0.02</td>
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<tr>
<td>CV (%)</td>
<td>1.5</td>
<td>1.9</td>
</tr>
</tbody>
</table>

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Appendix 4: Blood lactate assay

Blood lactate was measured using a modification of the enzymatic method described by Maughan (1982). Samples were assayed in duplicate.

**Principle.**

\[
\text{LDH} \\
\text{lactate + NAD}^+ \rightarrow \text{pyruvate + NADH + H}^+
\]

NAD = nicotinamide-adenine-dinucleotide  
LDH = lactate dehydrogenase

**Deproteinisation.**

20 µl of blood was deproteinised in 200 µl of perchloric acid (2.5%). The sample was then mixed thoroughly, centrifuged and stored at -70 ºC until required.

**Solutions.**

Perchloric acid (2.5%): 10.4 ml of 60% perchloric acid made up to 250 ml with distilled water gave a 2.5% perchloric acid solution.

Hydrazine buffer (pH 9.1): 6.5 g hydrazinium sulphate; 24.26 ml hydrazine hydrate; and 1 g ethylenediaminetetra-acetic acid (EDTA) made up to 500 ml with distilled water.

Lactate diluent (0.07 mole hydrochloric acid): this was made up from a 1 mole solution of hydrochloric acid (86 ml of hydrochloric acid, specific gravity 1.18, made up to 1 litre with distilled water). 70 ml of 1 mole hydrochloric acid was made up to 1 litre with distilled water giving a 0.07 mole hydrochloric acid solution.

Reaction mixture: prepared immediately before use: 2 mg of NAD and 10 µl of LDH per ml of hydrazine buffer.
Standards.

These were made up from 1.0 mole lactate stock and included the following concentrations: 2.5; 5.0; 7.5; 10.0 and 15.0 mmol.l⁻¹.

Procedure.

1. Samples were allowed to warm up to room temperature for at least one hour.

2. Samples were mixed thoroughly in a vortex.

3. Samples were centrifuged for four minutes at 12,000 rpm.

4. 20 µl of supernatant or standard was pipetted into a glass test tube.

5. 200 µl of reaction mixture was added to each test tube.

6. Tubes were vortexed and left to incubate for 30 minutes.

7. 1 ml of lactate diluent was added to the test tubes which were again vortexed.

8. The fluorescence of the samples and standards was determined using a Locarte fluorimeter. Lactate diluent was used as a blank.

9. A linear regression equation was calculated using the known standard concentrations and their respective fluorescence values. The concentration of lactate in the samples was ascertained using this regression equation.
Appendix 5: Haemoglobin assay

Haemoglobin concentration was determined using a test kit. Samples were assayed in duplicate.

Kit: Test - Combination Haemoglobin.
Supplier: Boehringer Mannheim, U.K. Ltd.

Principle.

\[
\text{haemoglobin} + \text{cyanide} + \text{ferricyanide} \rightarrow \text{cyanmethemoglobin}
\]

Solution.

Drabkin's solution: one bottle of potassium hexacyanoferrate(III) solution and one bottle of potassium cyanide solution (both supplied with the kit) were dissolved in distilled water and made up to 1 litre in a graduated cylinder.

Procedure.

1. 20 µl of whole blood was collected into micro-pipettes and immediately diluted into 5 ml of Drabkin's solution.

2. The samples were shaken vigorously.

3. The absorbance of the samples was measured at a wavelength of 546 nm using an Eppendorf photometer (model 1101 M). Drabkins' solution was used as a blank.

4. Haemoglobin concentration (g.100 ml^{-1}) was determined by multiplying the absorbance value of the sample by 36.77.
Appendix 6: Total cholesterol assay

Total cholesterol concentration was determined from serum samples by an enzymatic colourimetric method using a commercially available kit. Samples were assayed in duplicate on a Cobas Mira automated analyser.

Kit: Cholesterol C-system, CHOD-PAP method.
Standards: Preciset Cholesterol.
Quality control: Precinorm L.
Supplier: Boehringer Mannheim, U.K. Ltd.

Principle.

\[
\text{cholesterol esterase} \\
\text{cholesterol ester} + H_2O \rightleftharpoons \text{cholesterol} + RCOOH
\]

\[
\text{cholesterol oxidase} \\
\text{cholesterol} + O_2 \rightleftharpoons \Delta^4\text{-cholestenone} + H_2O_2
\]

\[
\text{POD} \\
2 H_2O_2 + 4\text{-aminophenazone} + \text{phenol} \rightarrow 4\text{-}(p\text{-benzoquinone-monoimino})\text{-phenazone} + 4 H_2O
\]

Solution.

Bottles of reagent solution supplied were dissolved in distilled water and made up to 32 ml.

Procedure.

1. Samples were removed from the freezer and allowed to warm to room temperature.
2. Samples were mixed in a vortex, dispensed into plastic tubes and loaded onto the Cobas Mira analyser.
3. 5 μl of sample or control sera was pipetted into a cuvette.
4. 350 μl of reagent was added to each cuvette.
5. Samples were mixed and left to incubate for 7.5 minutes.

6. The absorbance of the samples was read at a wavelength of 500 nm.

7. Sample concentrations were calculated by the Cobas Mira analyser using a regression equation derived from a reagent blank and one point standard (Precinorm L).

NB During the storage study (Section 3.11) the above assay was performed manually at Loughborough University. In this case the procedures varied slightly as follows. The volumes of sample and reagent were 10 μl and 1 ml respectively. The incubation period was 30 minutes. Samples were read on an Eppendorf photometer (model 1101 M) at a wavelength of 546 nm. Absorbance values from Preciset cholesterol standards were paired with the respective known concentrations of the standards to calculate a linear regression equation. Sample concentrations were derived from this equation.
Appendix 7: Precipitation procedures for the determination of HDL-C and HDL₃-C

Serum samples requiring precipitation were stored at 4 °C after collection. Precipitation was performed within five days of sample collection. Further details of the precipitation procedures used have been reported elsewhere (Farish and Fletcher, 1983; Warnick and Albers, 1978).

Principle.

An insoluble complex is formed between a polyanion-divalent cation and lipoprotein particles. Manganese and heparin selectively precipitate chylomicrons, VLDL and LDL leaving HDL in solution. Following centrifugation, HDL is measured in the supernatant.

Solutions.

Sodium Heparin: this solution was made up fresh each day using Sigma grade I sodium salt from porcine intestinal mucosa which was dissolved in 1 ml of 9 g.l⁻¹ saline. The weight of sodium heparin used depended on the number of USP units as follows:

- 181 USP units = 31.5 mg per ml of saline
- 178 USP units = 32.0 mg per ml of saline
- 176 USP units = 32.4 mg per ml of saline

Manganese Chloride (1 mol.l⁻¹): 4.95 g of MnCl₂.4H₂O were dissolved in 25 ml of distilled water in a volumetric flask.

EDTA (Ethylenediaminetetra-acetic acid) (0.4 mol.l⁻¹): 14.88 g of EDTA, disodium salt, was dissolved in 100 ml of distilled water. Solubilisation of the EDTA was achieved by adjusting to pH 7.7 while stirring.

Dextran sulphate (15.4 g.l⁻¹): 77 mg of dextran sulphate was dissolved in 5 ml of 9 g.l⁻¹ saline. This solution was only required if HDL subfractions were being measured.
Procedure for HDL-C determination.

1. 40 μl of sodium heparin solution was added to 1 ml of serum.
2. The sample was immediately mixed in a vortex for ten seconds.
3. 100 μl of manganese chloride was added to the sample.
4. The sample was again mixed in a vortex for ten seconds.
5. The sample was incubated in an ice bath at 4 °C for 30 minutes.
6. The sample was centrifuged (4 °C) for 25 minutes at 4,000 rpm.
7. The supernatant was removed and stored at -70 °C until required for total HDL analysis (Appendix 8) or for further precipitation of HDL₂ cholesterol as described below. If precipitant was floating the supernatant was removed using a Millipore filter.

Procedure for HDL₂-C determination.

1. 50 μl of dextran sulphate was added to 500 μl of the thawed heparin/ manganese chloride supernatant.
2. The sample was mixed in a vortex for five seconds.
3. The sample was left to stand for 20 minutes at room temperature.
4. The sample was centrifuged (4 °C) for 30 minutes at 4,000 rpm.
5. The supernatant was removed and used for HDL₃-C determination as described in Appendix 8.

NB Problems were experienced in the precipitation of HDL₂-C from the serum samples obtained during the brisk walking study. In the event no reliable information was gained from the brisk walking study regarding HDL subfractions. A study (Section 3.11) was conducted to investigate whether or not the problems in HDL-C subfraction measurement were caused by storage. In this study there were slight variations to the above procedures (see Section 3.11.2).
Appendix 8: HDL-C and HDL$_3$-C assay

Procedure.

1. Precipitated samples were removed from the freezer and given at least one hour to warm to room temperature.

2. Samples were mixed in a vortex.

3. 250 µl of supernatant was added to 10 µl of EDTA.

4. Samples were again mixed in a vortex and left to stand for five minutes.

5. The procedure for total cholesterol (Appendix 6) was then followed using 15 µl of sample or, in the case of the storage study, 20 µl of sample.

6. The concentrations of HDL-C and HDL$_3$-C derived from the cholesterol assay were multiplied by correction factors as follows: 1.1856 x HDL-C concentration and 1.2896 x HDL$_3$-C concentration. These correction factors were required to adjust for dilution of the samples by heparin, manganese chloride and dextran sulphate.

7. HDL$_2$-C concentration was calculated as HDL-C - HDL$_3$-C.
Appendix 9: Triglyceride assay

Triglyceride concentration was determined from serum samples by an enzymatic colourimetric method using a commercially available kit. All samples were assayed in duplicate on a Cobas Mira automated analyser.

Kit: Triglycerides GPO-PAP
Quality control: Precinorm L.
Supplier: Boehringer Mannheim, U.K. Ltd.

Principle.

\[ \text{lipase} \]
\[ \text{triglycerides} + 3 \text{H}_2\text{O} \rightarrow \text{glycerol} + 3 \text{RCOOH} \]

\[ \text{GK} \]
\[ \text{glycerol} + \text{ATP} \rightarrow \text{glycerol-3-phosphate} + \text{ADP} \]

\[ \text{GPO} \]
\[ \text{glycerol-3-phosphate} + \text{O}_2 \rightarrow \text{dihydroxyacetone phosphate} + \text{H}_2\text{O}_2 \]

\[ \text{peroxidase} \]
\[ \text{H}_2\text{O}_2 + 4\text{-aminophenazone} + 4\text{-chlorophenol} \rightarrow 4\text{-}(p\text{-benzoquinone-mono-imino})\text{-phenazone} + 2 \text{H}_2\text{O} + \text{HCL} \]

Solutions.

Buffer solution
Reagent strips

Both of the above were supplied with the kit. A reagent strip was immersed in a bottle of buffer solution, stirred for ten seconds and left for five minutes. The buffer was again stirred with the reagent strip for ten seconds and the strip was discarded.
Procedure.

1. Serum samples were removed from the freezer and given at least one hour to thaw.

2. Samples were mixed in a vortex, dispensed into plastic tubes and loaded onto the Cobas Mira analyser.

3. 5 µl of sample or control sera was pipetted into cuvettes.

4. 350 µl of reaction mixture was added to the cuvettes.

5. The samples were mixed and left to incubate for 7.5 minutes.

6. The samples were read at 500 nm.

7. Sample concentrations were calculated by the Cobas Mira analyser using a regression equation derived from a reagent blank and one point standard (Precinorm L).
Appendix 10: Calculation of LDL-C and VLDL-C

Friedewald and colleagues (1972) devised the following formula to calculate LDL-C and VLDL-C (mg.dl\(^{-1}\)):

\[
\text{triglyceride concentration} + 5 = \text{VLDL-C}
\]

\[
\text{LDL-C} = \text{total cholesterol} - \text{HDL-C} - \text{VLDL-C}
\]

This formula is based on two observations. One is that the ratio of the mass of triglyceride to that of cholesterol in VLDL is relatively constant at about 5:1 in normal subjects. The other is that when chylomicrons are not detectable (as is the case in serum from fasted subjects), most of the triglyceride in plasma is contained in VLDL.

If values are expressed in mmol.l\(^{-1}\) (as in this thesis) then triglyceride concentration is not divided by 5 but by 2.2 to obtain VLDL-C.
Appendix 11: Assay for Apoproteins A-I and B

Apoproteins A-I and B were determined from serum samples by immunoprecipitin analysis. Measurements were made on a Cobas Mira automated analyser using a commercially available kit.

Kit: Antibody reagent set II for Apo A-I or Apo B.

Standards: Calibration set for Apo A-I or Apo B, concentrations as follows:
Apo A-I: 0.26, 0.65, 1.30, 1.96, 2.61 g.l\(^{-1}\).
Apo B: 0.29, 0.72, 1.44, 2.15, 2.87 g.l\(^{-1}\).

These standards were calibrated against Centre for Disease (CDC) reference material.

Supplier: Atlantic Antibodies
Stillwater
Minnesota
55082 USA.

Principle.

A microvolume of sample (antigen) is diluted and mixed with antiserum (antibody) in a reaction cuvette. Insoluble antigen-antibody complexes form producing turbidity in the mixture and thus increasing the amount of light scattered by the solution. The absorbance of the solution is then measured at the analytical wavelength. A calibration curve is generated by assaying a series of standards. The concentration of apoprotein in the sample is interpolated from the calibration curve.

Solutions. (supplied with kit)

Antibody reagent.
Antibody diluent: Apo A-I antibody diluent for Apo A-I
Polymer diluent for Apo B.
Diluent A.
Procedure.

1. Samples were removed from the freezer and allowed to warm to room temperature.

2. Samples were mixed in a vortex.

3. Antibody reagent was diluted 1:21 in antibody diluent (1 part antibody reagent plus 20 parts antibody diluent).

4. Standards, controls and samples were diluted 1:21 in diluent A (1 part sample and 20 parts diluent A). These were then mixed thoroughly and loaded onto the Cobas Mira analyser.

5. 5 (Apo A-I) or 10 (Apo B) μl volumes of sample were mixed with 250 μl volumes of reagent and incubated for eight minutes.

6. Samples were read at 340 nm.

7. A calibration curve was developed by the Cobas Mira using the absorbances of the standards and their respective assigned concentrations. Sample concentrations were calculated from this calibration curve.
Appendix 12: Lp(a) assay

Lp(a) concentration was determined from serum samples using an enzyme linked immunosorbant assay (ELISA).

Kit: Enzyquick Lp(a).

Supplier: Immuno Ltd.
Artic House
Dunton Green
Sevenoaks
Kent.

Additional equipment.

Titertek multi-scan plate reader (model MCC/340)
Titertek washer (model S8/12)
Both of the above were purchased from: ICN Flow
Highwickham
Buckinghamshire.

Principle.

During an initial incubation period Lp(a) from the sample binds to Lp(a) antibodies which have been coated onto the surface of test strip wells. Non specifically bound protein material is subsequently removed from the wells during a washing procedure.

In a second incubation step anti-Lp(a) antibodies conjugated to the enzyme peroxidase bind to the Lp(a) fixed to the solid phase antibody in the wells. A further washing step removes unbound conjugate and then the peroxidase enzyme substrate TMB (3,3,5,5, Tetramethylbenzidine) is added. The substrate produces a colour after reacting with the conjugated second antibody. After stopping the reaction with sulphuric acid the amount of colour produced is measured (450 nm) and will be directly proportional to the Lp(a) concentration in the sample.
Kit contents.

Twelve test strips containing eight wells each coated with Lp(a) antibodies.
Two vials of peroxidase conjugated anti-Lp(a). Lyophilised.
One vial of Lp(a) negative human control serum. Lyophilised.
Six vials of Lp(a) reference standards (5, 10, 20, 40, 60, 80 mg.dl\(^{-1}\)). Lyophilised.
One vial of buffer concentrate for samples and conjugate.
One vial of substrate TMB (3,3,5,5, Tetramethylbenzidine) liquid: 500 μl.
Two vials of substrate dilution buffer, 12 ml each.
One vial of 4N H\(_2\)SO\(_4\) (sulphuric acid), 5.5 ml.

Reconstitution of reagents.

Buffer concentrate: 100 ml diluted in 1 litre of distilled water.

Peroxidase conjugated anti-Lp(a): reconstituted with 1.2 ml of buffer concentrate. Reconstituted contents were then diluted 1 + 10 with buffer concentrate.

Lp(a) reference sera: reconstituted in 200 μl of distilled water and left to stand for 30 minutes.

Lp(a) negative control serum: reconstituted in 1 ml of distilled water and left to stand for 30 minutes.

Substrate (TMB) solution: 40 μl of substrate solution was diluted in 2 ml of substrate buffer per eight tests.

Procedure.

1. Samples were removed from the freezer and allowed to warm to room temperature.

2. Samples were mixed in a vortex.

3. The standards and samples were diluted 1 : 500 with buffer concentrate using a two step dilution as follows. 100 μl of standard or serum was
added to 1 ml of buffer concentrate, then 100 μl of this diluted standard or serum was added to 5 ml of buffer concentrate.

4. Standard and sample dilutions were mixed thoroughly.

5. 200 μl of negative control serum was pipetted into the first four wells of the first test strip.

6. 200 μl of each standard was pipetted in duplicate into the remaining wells of test strip one and test strip two.

7. 200 μl of each sample was pipetted into a test well starting from strip three.

8. The test strips were covered with foil and incubated for one hour.

9. The test strips were emptied and washed five times with the buffer concentrate solution using a Titertek washer. After the final wash, remaining liquid was removed by inverting the strips and tapping them on absorbant material.

10. 200 μl of peroxidase conjugated anti-Lp(a) was pipetted into each well of the test strip and incubated for one hour.

11. The test strips were emptied and washed as above.

12. 200 μl of substrate solution was added to each well of the test strips which were then incubated for ten minutes.

13. 50 μl of sulphuric acid was added to each well to stop the enzymatic reaction.

14. The absorbances were read at 450 nm using a Titertek multi-scan plate.

15. A reference curve was produced by plotting the absorbance of the standards against their known concentrations. The Lp(a) concentration of the samples was calculated by reading their absorbance values on the reference curve.
Appendix 13: Between and within batch precision on the Cobas Mira analyser

<table>
<thead>
<tr>
<th></th>
<th>Between batch precision</th>
<th>Within batch precision</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
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<tr>
<td>Cholesterol (mmol.l⁻¹)</td>
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<td>0.11</td>
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<tr>
<td></td>
<td>6.8</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>10.3</td>
<td>0.31</td>
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<tr>
<td>Triglyceride (mmol.l⁻¹)</td>
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<td>0.02</td>
</tr>
<tr>
<td></td>
<td>1.3</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>3.9</td>
<td>0.09</td>
</tr>
<tr>
<td>HDL-C (mmol.l⁻¹)</td>
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<td>0.05</td>
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<tr>
<td></td>
<td>2.27</td>
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</tr>
<tr>
<td>Apo A-I (g.l⁻¹)</td>
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<td>0.02</td>
</tr>
<tr>
<td></td>
<td>1.46</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>2.10</td>
<td>0.05</td>
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<tr>
<td>Apo B (g.l⁻¹)</td>
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<td></td>
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<td>0.02</td>
</tr>
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</table>
Appendix 14: Fibrinogen assay

Plasma fibrinogen concentration was measured via a coagulation assay using a commercially available kit.

Kit: Multifibren.
Supplier: Behring
P.O. Box 1140
Marburg
W. Germany.

Principle.

Citrated plasma is clotted by the addition of thrombin. The clotting time is measured and will depend primarily on the fibrinogen content of the blood sample.

Plasma separation.

Two millilitre samples of blood were poured into citrated tubes (Sarstedt 9NC/2) and centrifuged for 15 minutes at 6,000 rpm in a koolspin (4 °C). The plasma was then removed using pasteur pipettes and dispensed into a 1 ml plastic tubes. These were frozen (-70 °C) until required for analysis.

Solutions.

Multifibren: lyophilised alpha-thrombin from bovine plasma (supplied with kit).

Control plasma: lyophilised human plasma (supplied with kit).

Barbital buffer solution (pH 7.35): 11.75 g of sodium diethyl barbiturate and 14.67 g of sodium chloride were dissolved in a mixture of 1570 ml of distilled water and 430 ml of 100 mmol.l⁻¹ hydrochloric acid.

Kaolin suspension: this was purchased separately from Behring.
Reconstitutions.

Multifibren: 10 ml of multifibren was reconstituted with 5 ml of kaolin suspension and left to stand for ten minutes.

Control Plasma: one vial of control plasma was dissolved in 1 ml of distilled water and allowed to stand for 15 minutes.

Procedure.

1. Plasma was diluted 1:9 with barbital buffer solution (0.1 ml of plasma and 0.9 ml of buffer). In the case of plasma samples with very low or very high fibrinogen concentrations different dilutions were used (i.e. 1 + 4 and 1 + 19 respectively).

2. 0.1 ml of reconstituted multifibren was added to 0.2 ml of citrated plasma dilution.

3. Clotting time was measured on a fibrintimer (Behring).

4. Clot time (seconds) was converted to fibrinogen concentration (mg.dl⁻¹) using a table of values enclosed with the kit. The values on these tables applied only to samples diluted 1:9. For samples diluted 1:4 or 1:19 the tabular values were multiplied by correction factors as follows:

   Factor 0.5 for 1:4 dilutions
   Factor 2 for 1:19 dilutions
Appendix 15: One page example of the training diaries used by the walkers

BRITISH HEART FOUNDATION STUDY
LOUGHBOROUGH UNIVERSITY
TRAINING DIARY

Name ............................

Fortnight beginning .......................... 1989/90

<table>
<thead>
<tr>
<th>Day</th>
<th>Minutes of brisk walking</th>
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<td>Mon</td>
<td></td>
</tr>
<tr>
<td>Tues</td>
<td></td>
</tr>
<tr>
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<tr>
<td>Sun</td>
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</table>

Fortnightly total (mins) ............
Fortnightly target (mins) ............

Comments: