High-carbohydrate diets, exercise and postprandial lipaemia

This item was submitted to Loughborough University’s Institutional Repository by the/an author.

Additional Information:


Metadata Record: https://dspace.lboro.ac.uk/2134/35001

Publisher: C. Koutsari

Rights: This work is made available according to the conditions of the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) licence. Full details of this licence are available at: https://creativecommons.org/licenses/by-nc-nd/4.0/

Please cite the published version.
HIGH-CARBOHYDRATE DIETS, EXERCISE AND POSTPRANDIAL LIPAEMIA

by

Christina Koutsari

A Doctoral Thesis

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

October 2000

© by C. Koutsari (2000)
ABSTRACT

Low-fat, high-carbohydrate diets are recommended by various scientific bodies for the prevention of coronary heart disease. However, these diets increase postprandial lipaemia and so their net benefit for coronary heart disease risk has been the subject of vigorous debate. Exaggerated postprandial lipaemia has been implicated in the development of atherosclerosis. Previous research suggests that physical exercise improves triacylglycerol metabolic capacity. The present thesis investigated whether exercise, when simultaneously adopted with a high-carbohydrate diet, could prevent the augmentation of postprandial lipaemia observed with this dietary change.

The aims of the first study were to establish a three-day dietary intervention model and to examine the effects of two extreme isoenergetic diets (high-fat versus high-carbohydrate) on the triacylglycerol response to a standard test meal. In the group of healthy young men studied, postprandial lipaemia (expressed as the area under the plasma triacylglycerol concentration vs time curve) was 90% higher after the three-day high-carbohydrate diet than after the three-day high-fat diet, with major differences in the associated metabolic milieu.

The second study tested the hypothesis that 30 minutes of daily moderate intensity exercise offsets the augmentation of postprandial lipaemia associated with a high-carbohydrate diet. Postprandial lipaemia was 35% greater after subjects had followed a three-day high-carbohydrate diet, while refraining from exercise, than after they had followed a typical Western diet. However, the addition of daily exercise to the high-carbohydrate diet significantly reduced postprandial lipaemia, almost to the level observed after the Western diet.

The third study aimed to provide a detailed description of the qualitative changes to postprandial triacylglycerol-rich lipoprotein metabolism elicited by a high-carbohydrate diet and moderate intensity exercise in a group of healthy postmenopausal women. The postprandial triacylglycerol response in the triacylglycerol-rich lipoprotein fraction was 32% greater after a three-day high-carbohydrate diet than after a three-day low-carbohydrate diet. This was associated with increases in the numbers of apolipoprotein B48-, apolipoprotein B100-containing triacylglycerol-rich lipoproteins and their remnants. When subjects had exercised for 60 minutes daily whilst consuming the high-carbohydrate diet, none of the above parameters differed significantly from the low-carbohydrate diet.

In conclusion, regular moderate exercise is sufficient to prevent the augmentation of postprandial lipaemia and accumulation of triacylglycerol-rich lipoproteins attributable to a short-term high-carbohydrate diet. Regular exercise may provide one means for allaying current anxieties about high-carbohydrate diets.
ACKNOWLEDGEMENTS

I am grateful to the Greek State Scholarships Foundation for generously supporting me during my doctoral training. I am also indebted to the British Heart Foundation for funding the research described in chapter 6.

I would also like to express my gratitude to the following people:

My Supervisor, Professor Adrianne Hardman, for providing me with the opportunity to undertake the research presented in the following pages, and for her guidance, advice and valuable discussion throughout my doctoral training. Her assistance during the busy experimental days is also gratefully acknowledged.

My Director of Research, Professor Clyde Williams, for his general advice and encouragement throughout my PhD.

Dr Dalé Malkova for sharing inspired thoughts and her support over the past three years but above all, for her friendship.

Professor Keith Frayn from the Oxford Lipid Metabolism Group for his interest in my research project and giving me the opportunity to collaborate with his group. The use of his laboratory proved invaluable, as was his advice and help with the design and interpretation of the results.

Dr Fredrik Karpe from the Oxford Lipid Metabolism Group for introducing me to the methods for lipoprotein fractionation and quantification of apolipoproteins and for his invaluable contribution and intellectual help with my research. His positive attitude and friendship have pushed me forward.

The other members of the Oxford Lipid Metabolism Group for their help and accommodating nature during my time in their laboratories. Especially, Mrs Sandy Humphreys for her technical assistance with analytical work and statistical advice and Mrs Carine Beysen for her friendship.

Professor Peter Jones for all his help during the busy experimental days. His sense of humour and gentle manners towards the participants created a very pleasant atmosphere to work in.

Miss Margaret Derblay, Miss Jane Riley and Miss Julia Wells for their help with the data collection.

Ms Lisa Pierce for performing the dietary analyses and prescription in the study described in chapter 6.
Dr Sarabjeet Mastana and Mrs Alice Pacynco for measurements of apolipoprotein E phenotypes.

Mr Spencer Newport and Miss Gabriella Falero for ordering the chemicals needed during the busy experimental periods.

Mr Ramesh Mistry for valuable technical assistance with software and hardware.

All the other members of the Human Muscle Metabolism Research Group, especially PhD students, for their help over the past three years.

Professor Christos Kambitsis, who was my supervisor on behalf of the Greek State Scholarships Foundation.

Dr Vassilis Mougios for introducing me to biochemistry of exercise in a very pleasant way and initiating my interest in human exercise metabolism.

All the students in Cayley Hall, the other Subwardens and the Wardens, Professor Ian Henry and Mrs Susan Reed for creating a delightful atmosphere to live in and not letting me forget how great student life is.

All my friends within and outside university life for their support and friendship.

My family for their continuing support and confidence in me, especially my mother. Her strength, courage and love have helped me to achieve many, if not all the worthwhile things I have done.

Dimitri for his understanding and encouragement all these years and making me smile even in my darkest hour.

Finally, all the participants of the studies described in this thesis for giving their time and energy so freely. Without their efforts to follow the diets, exercise and eat the creamy meal, this thesis would not have been possible.
“Live as if you were to die tomorrow.  
Learn as if you were to live forever.”  

Mahatma Gandhi
PART OF THE WORK PRESENTED IN THIS THESIS HAS BEEN PUBLISHED AS FOLLOWS:

**ORIGINAL ARTICLES**


**PUBLISHED COMMUNICATIONS**


TABLE OF CONTENTS

CHAPTER 1
INTRODUCTION 1
AIMS OF THESIS 6

CHAPTER 2
REVIEW OF LITERATURE 7

2.1 Introduction 7
2.2 Lipids and lipoproteins 7
2.3 Metabolism of postprandial lipid and TRL particles 10
   2.3.1 Metabolism of chylomicrons and their remnants: the exogenous pathway 10
   2.3.2 Metabolism of VLDL and their remnants: the endogenous pathway 13
   2.3.3 Integration of TRL metabolism in the postprandial state 14
2.4 Regulation of postprandial TAG metabolism 17
   2.4.1 Insulin 17
   2.4.2 Lipoprotein lipase 18
2.5 Factors affecting postprandial lipaemia 19
   2.5.1 Postabsorptive plasma TAG concentration 19
   2.5.2 Preceding diet 20
   2.5.3 Age and gender 21
2.6 Methods for studying TAG and TRL metabolism 22
   2.6.1 Oral fat tolerance test 22
   2.6.2 Intravenous fat tolerance test 23
   2.6.3 Oral retinyl ester assay 23
   2.6.4 Direct measurement of apo B48 and apo B100 in the TRL fraction 24
2.7 Postprandial TAG metabolism and atherosclerosis 25
   2.7.1 Postprandial increase in TRL particles and their remnants 26
   2.7.2 Low HDL-cholesterol 30
   2.7.3 Small dense LDL particles 32
2.8 Effect of low-fat, high carbohydrate diets on TAG metabolism 33
   2.8.1 Effect of high-carbohydrate diets on fasting TAG concentration 33
2.8.2 Effect of high-carbohydrate diets on postprandial TAG concentration 35
2.8.3 Factors affecting TAG response to a high-carbohydrate diet 38
  2.8.3.1 Energy content of the high-carbohydrate diet 39
  2.8.3.2 Duration of the high-carbohydrate diet 40
  2.8.3.3 Carbohydrate composition of the high-carbohydrate diet 42
2.8.4 Mechanisms for carbohydrate-induced increases in TAG concentration 43
  2.8.4.1 Hepatic overproduction of VLDL-TAG 43
  2.8.4.2 Defective hydrolysis of TRL-TAG 46
2.9 Effect of exercise on TAG metabolism 48
  2.9.1 Effect of exercise on fasting TAG concentration 49
  2.9.2 Effect of exercise on postprandial TAG concentration 50
    2.9.2.1 Effect of exercise training 50
    2.9.2.2 Effect of a single exercise session 53
  2.9.3 Mechanisms for exercise-induced decreases in TAG concentration 56
    2.9.3.1 Enhanced hydrolysis of TRL-TAG 56
    2.9.3.2 Reduced hepatic production of VLDL-TAG 59
2.10 Summary 59

CHAPTER 3
GENERAL METHODS 61
3.1 Subjects 61
3.2 Preliminary recording and testing 62
  3.2.1 Diet recording 62
  3.2.2 Exercise tests 62
    3.2.2.1 Submaximal exercise testing 62
    3.2.2.2 Maximal exercise testing 64
3.3 Measurement of oxygen uptake and carbon dioxide production 65
3.4 Heart rate 66
3.5 Perceived rate of exertion 66
3.6 Anthropometry 66
  3.6.1 Body Mass Index 67
  3.6.2 Waist and hip circumferences 67
3.6.3 Percentage body fat - skinfold thickness  68
3.7 Diet analysis  68
3.8 Experimental design and main interventions  69
   3.8.1 Experimental diets  70
   3.8.2 Exercise sessions  71
   3.8.3 Preparation for the main interventions  71
3.9 Test meal protocol  72
   3.9.1 The test meal  72
   3.9.2 Preparation for the test meal  75
3.10 Blood sampling  75
3.11 Analysis of blood samples  76
   3.11.1 Spectrophotometric assays  76
   3.11.2 Radioimmunoassays  76
   3.11.3 Apolipoprotein E phenotyping  77
   3.11.4 TRL fractionation  77
   3.11.5 Determination of TRL apo B48, apo B100 and apo E concentrations  78
   3.11.6 Determination of remnant-like lipoprotein particle cholesterol concentration  80
3.12 Calculations and statistics  83
3.13 Accuracy and precision  84

CHAPTER 4
POSTPRANDIAL LIPAEMIA AFTER SHORT-TERM VARIATION IN DIETARY FAT AND CARBOHYDRATE  85
4.1 Introduction  85
4.2 Subjects and methods  87
   4.2.1 Subjects  87
   4.2.2 Study design  89
   4.2.3 Experimental diets  89
   4.2.4 Diet analysis  89
   4.2.5 Test meal protocol  90
4.2.6 Analytical methods 90
4.2.7 Calculations and statistics 94

4.3 Results 94
4.3.1 Experimental diets and test meal 94
4.3.2 Plasma and serum concentrations in the fasted state 94
4.3.3 Postprandial responses 94

4.4 Discussion 103

CHAPTER 5
EXERCISE PREVENTS THE AUGMENTATION OF POSTPRANDIAL LIPEMIA ATTRIBUTABLE TO A LOW-FAT, HIGH-CARBOHYDRATE DIET 109

5.1 Introduction 109
5.2 Subjects and methods 111
5.2.1 Subjects 111
5.2.2 Study design 113
5.2.3 Preliminary exercise tests 113
5.2.4 Experimental diets 113
5.2.5 Exercise sessions 118
5.2.6 Test meal protocol 118
5.2.7 Diet analysis 119
5.2.8 Analytical methods 119
5.2.9 Calculations and statistics 119

5.3 Results 120
5.3.1 Experimental diets and test meal 120
5.3.2 Cardiorespiratory and metabolic responses during exercise sessions 120
5.3.3 Plasma and serum concentrations in the fasted state 120
5.3.4 Postprandial responses 122
5.3.5 Indirect calorimetry 128

5.4 Discussion 130
Appendix A2 – Statement of informed consent 207
Appendix A3 – Health history questionnaire for study volunteers 208
Appendix A4 – Physical activity questionnaire 210
Appendix A5 – Food inventory form 211
Appendix A6 – Food inventory instructions 212
Appendix B1 – Determination of plasma and TRL-TAG concentration with correction for free glycerol 215
Appendix B2 – Determination of serum insulin concentration 219
LIST OF TABLES

2.1 Characteristics of the major lipoprotein classes 9
3.1. Quantities of ingredients of the test meal, administered per kg body mass 73
3.2. Macronutrient composition of the test meal 73
3.3. Energy and macronutrient content of test meal for an example subject 74
4.1 Physical characteristics of subjects and plasma concentrations of lipoprotein lipids measured in the fasted state 88
4.2 Energy content and composition of the habitual and experimental (high-carbohydrate and high-fat) diets 91
4.3 An example of the prescribed high-carbohydrate diet 92
4.4 An example of the prescribed high-fat diet 93
4.5 Concentrations measured in the fasted state for plasma TAG, total cholesterol, HDL cholesterol, non-esterified fatty acids, glucose, lactate, and serum 3-hydroxybutyrate and insulin, after the high-carbohydrate diet and after the high-fat diet 96
4.6 Six-hour areas under the plasma or serum concentration vs time curves for TAG, non-esterified fatty acids, 3-hydroxybutyrate, insulin, glucose and lactate after three days on a high-carbohydrate diet and after three days on a high-fat diet 102
5.1 Physical characteristics of subjects and plasma concentrations of lipoprotein lipids measured in the fasted state 112
5.2 Energy content and composition of the habitual and experimental (typical Western and high-carbohydrate) diets 115
5.3 An example of the prescribed typical Western diet 116
5.4 An example of the prescribed high-carbohydrate diet 117
5.5 Plasma or serum concentrations measured in the fasted state after three days on a typical Western diet (Western), after three days on a high-carbohydrate diet (High-CHO) and after three days on the same high-carbohydrate diet with the addition of 30 minutes daily moderate intensity exercise (High-CHO-Ex) 121
5.6 Six-hour areas under the plasma or serum concentration vs time curves for TAG, non-esterified fatty acids, 3-hydroxybutyrate, insulin, glucose and lactate after three days on a typical Western diet (Western), after three days on a high-
carbohydrate diet (High-CHO) and after three days on the same high-carbohydrate diet with 30 minutes daily of moderate exercise (High-CHO-Ex) 127

6.1 Physical characteristics of subjects and plasma concentrations of lipoprotein lipids measured in the fasted state 136

6.2 Energy content and composition of the habitual and experimental (low- and high-carbohydrate) diets 139

6.3 An example of the prescribed low-carbohydrate diet 140

6.4 An example of the prescribed high-carbohydrate diet 141

6.5 Plasma and TRL fraction concentrations of lipids and apolipoproteins measured in the fasted state after the low-carbohydrate diet (Low-CHO), after the high-carbohydrate diet (High-CHO), and after the high-carbohydrate diet with the addition of 60 minutes daily moderate intensity exercise (High-CHO-Ex) 147

6.6 Plasma non-esterified fatty acids and glucose and serum 3-hydroxybutyrate and insulin concentrations measured in the fasted state after three days on a low-carbohydrate diet (Low-CHO), after three days on a high-carbohydrate diet (High-CHO), and after three days on the same high-carbohydrate diet with the addition of 60 minutes daily moderate intensity exercise (High-CHO-Ex) 148

6.7 Six-hour areas under the plasma, TRL fraction or serum concentration vs time curves after three days on a low-carbohydrate diet (Low-CHO), after three days on a high-carbohydrate diet (High-CHO), and after three days on the same high-carbohydrate diet with the addition of 60 minutes daily moderate intensity exercise (High-CHO-Ex) 149

6.8 Remnant-like lipoprotein particle (RLP) cholesterol concentration in the fasted state (0 h) and at 4 h after the consumption of the test meal after three days on a low-carbohydrate diet (Low-CHO), after three days on a high-carbohydrate diet (High-CHO), and after three days on the same high-carbohydrate diet with the addition of 60 minutes daily moderate intensity exercise (High-CHO-Ex) 150
LIST OF FIGURES

4.1 Total (upper panel) and incremental (lower panel) plasma concentrations of TAG in the fasted state (0 hour) and for six hours following consumption of a high-fat, mixed meal after three days on a high-carbohydrate diet (High-CHO) and after three days on a high-fat diet (High-fat) 97

4.2 Serum concentrations of insulin in the fasted state (0 hour) and for six hours following consumption of a high-fat, mixed meal after three days on a high-carbohydrate diet (High-CHO) and after three days on a high-fat diet (High-fat) 99

4.3 Concentrations of plasma non-esterified fatty acids (NEFA) (upper panel) and serum 3-hydroxybutyrate (lower panel) in the fasted state (0 hour) and for six hours following consumption of a high-fat, mixed meal after three days on a high-carbohydrate diet (High-CHO) and after three days on a high-fat diet (High-fat) 100

4.4 Plasma concentrations of glucose (upper panel) and lactate (lower panel) in the fasted state (0 hour) and for six hours following consumption of a high-fat, mixed meal after three days on a high-carbohydrate diet (High-CHO) and after three days on a high-fat diet (High-fat) 101

5.1 Plasma TAG concentrations in the fasted state (0 hour) and for six hours following consumption of a high-fat mixed meal after three days on a typical Western diet (Western), after three days on a high-carbohydrate diet (High-CHO) and after three days on the same high-carbohydrate diet with 30 minutes daily of moderate exercise (High-CHO-Ex) 123

5.2 Serum insulin concentrations in the fasted state (0 hour) and for six hours following consumption of a high-fat mixed meal after three days on a typical Western diet (Western), after three days on a high-carbohydrate diet (High-CHO) and after three days on the same high-carbohydrate diet with 30 minutes daily of moderate exercise (High-CHO-Ex) 124

5.2 Plasma non-esterified fatty acids (NEFA) and serum 3-hydroxybutyrate concentrations in the fasted state (0 hour) and for six hours following consumption of a high-fat mixed meal after three days on a typical Western diet (Western), after three days on a high-carbohydrate diet (High-CHO) and after three days on the
same high-carbohydrate diet with 30 minutes daily of moderate exercise (High-CHO-Ex) 125

5.4 Plasma glucose and lactate concentrations in the fasted state (0 hour) and for six hours following consumption of a high-fat mixed meal after three days on a typical Western diet (Western), after three days on a high-carbohydrate diet (High-CHO) and after three days on the same high-carbohydrate diet with 30 minutes daily of moderate exercise (High-CHO-Ex) 126

5.5 Respiratory exchange ratio (RER) values in the fasted state (0 hour) and for six hours following consumption of a high-fat mixed meal after three days on a typical Western diet (Western), after three days on a high-carbohydrate diet (High-CHO) and after three days on the same high-carbohydrate diet with 30 minutes daily of moderate exercise (High-CHO-Ex) 129

6.1 Plasma TAG and TRL-TAG concentrations in the fasting state (0 hour) and for six hours following consumption of a high-fat mixed meal after three days on a low-carbohydrate diet (Low-CHO), after three days on a high-carbohydrate diet (High-CHO) and after three days on the same high-carbohydrate diet with the addition of 60 minutes daily moderate intensity exercise (High-CHO-Ex) 152

6.2 TRL-apo B48 and B100 concentrations in the fasted state (0 hour) and for six hours following consumption of a high-fat mixed meal after three days on a low-carbohydrate diet (Low-CHO), after three days on a high-carbohydrate diet (High-CHO) and after three days on the same high-carbohydrate diet with the addition of 60 minutes daily moderate intensity exercise (High-CHO-Ex) 153

6.3 TRL-apo E concentrations in the fasted state (0 hour) and for six hours following consumption of a high-fat mixed meal after three days on a low-carbohydrate diet (Low-CHO), after three days on a high-carbohydrate diet (High-CHO) and after three days on the same high-carbohydrate diet with the addition of 60 minutes daily moderate intensity exercise (High-CHO-Ex) 154

6.4 Serum insulin and plasma glucose concentrations in the fasted state (0 hour) and for six hours following consumption of a high-fat mixed meal after three days on a low-carbohydrate diet (Low-CHO), after three days on a high-carbohydrate diet (High-CHO) and after three days on the same high-carbohydrate diet with the addition of 60 minutes daily moderate intensity exercise (High-CHO-Ex) 155

6.5 Plasma non-esterified fatty acids (NEFA) and serum 3-hydroxybutyrate concen-
trations in the fasted state (0 hour) and for six hours following consumption of a high-fat mixed meal after three days on a low-carbohydrate diet (Low-CHO), after three days on a high-carbohydrate diet (High-CHO) and after three days on the same high-carbohydrate diet with the addition of 60 minutes daily moderate intensity exercise (High-CHO-Ex) 156

6.6 Respiratory exchange ratio (RER) values in the fasted state (0 hour) and for six hours following consumption of a high-fat mixed meal after three days on a low-carbohydrate diet (Low-CHO), after three days on a high-carbohydrate diet (High-CHO) and after three days on the same high-carbohydrate diet with the addition of 60 minutes daily moderate intensity exercise (High-CHO-Ex) 158
CHAPTER 1
INTRODUCTION

Coronary heart disease (CHD) is the leading cause of death in the Western society (Murray and Lopez 1997). The main determinant of CHD is atherosclerosis which is the formation of atheroma in the artery wall (Mangiapane and Salter 1999). Atherosclerosis involves accumulation of lipids in the intima of large elastic and muscular arteries. Initially these lipid deposits are known as fatty streaks. At some sites in the coronary arteries fatty streaks thicken by continuous, or intermittent, accumulation of lipid and connective tissue to form fibrous plaques or atheroma. Over years, these atheroma gradually increase in size and can undergo calcification and vascularisation. Such advanced atherosclerotic lesions can occlude arteries, reduce blood supply to the heart and produce severe clinical symptoms. Atherosclerosis begins in childhood but clinical manifestations may not occur until late adulthood. Both genetic and nongenetic factors are considered to play a causal role for the development of atherosclerosis. The nongenetic factors include cigarette smoking, hypertension, obesity, physical inactivity and abnormalities in lipid and lipoprotein metabolism.

Evidence – genetic, laboratory animals, clinical trials, epidemiologic – supports the proposition that high levels of low-density lipoprotein (LDL) cholesterol accelerate coronary atherosclerosis and that lowering of LDL-cholesterol levels decreases the risk of CHD (The Expert Panel 1988). Epidemiologic and experimental studies have demonstrated that saturated fatty acids consistently raise serum total (Keys et al. 1957; Hegsted et al. 1965; Grundy and Denke 1990; Caggiula and Mustad 1997) and LDL (Mensink and Katan 1992; Hegsted et al. 1993) cholesterol levels. Also, cross- and within-population studies have shown that intake of saturated fatty acids is strongly and positively associated with CHD incidence and mortality (Caggiula and Mustad 1997). This relation is generally believed to be mediated in part by effects of dietary saturated fat on total and LDL cholesterol concentrations and, for this reason, public health strategies are being developed and examined (Gibney 1999), aiming to reduce the consumption of saturated fat in the population. The existing dietary target in the United Kingdom is 33 % of the daily energy from fat, with only 10 % from saturated fatty acids (Department of Health 1991).
Despite the general acceptance of the atherogenic properties of saturated fatty acids, the desirable macronutrient replacement for saturated fat in the diet is an area of continuing debate. The two main contenders are complex carbohydrates and unsaturated fat. Replacement of saturated with polyunsaturated fat has been shown to reduce total and LDL cholesterol effectively (Mensink and Katan 1992; Katan 1997) and population studies have demonstrated that polyunsaturated fat intake is negatively associated with CHD mortality (Caggiula and Mustad 1997). As a result, early recommendations emphasised diets with a high ratio of polyunsaturated to saturated fat. However, the long-term safety of polyunsaturated fatty acids has not been confirmed by population data and, in the course of time, concerns arose over possible adverse effects, focusing initially on cancer risk and later also on the oxidisability of polyunsaturated fatty acids. Such considerations, along with epidemiologic evidence from the Mediterranean region where the diet contains large amounts of the monounsaturated oleic acid and CHD incidence is low (Keys et al. 1984), led researchers to favour replacement of saturated with monounsaturated fat. Indeed, recent reports have demonstrated that high-monounsaturated fatty acid diets efficiently lower total and LDL cholesterol levels (Mensink and Katan 1992; Kris-Etherton et al. 1999) and, therefore, these diets would be expected to reduce CHD risk.

However, distrust of the strategy to replace fat with fat (Connor and Connor 1997; Ornish 1998; Rudel 1998) and some, albeit inconsistent, epidemiologic evidence supporting the notion that high-fat diets promote the development of obesity (Seidell 1998) have favoured the replacement of saturated fat with carbohydrate, shifting the focus of recommendations to diets low in fat and high in carbohydrates. This approach was based on the fact that low-fat, high-carbohydrate diets have traditionally been consumed in Asia, and in this region, rates of CHD are low (Keys et al. 1984). Dietary fat restriction and consumption of high-carbohydrate diets have been widely recommended for the prevention of obesity (Schaefer et al. 1995), certain types of cancer (Butrum et al. 1988) and CHD (Krauss et al. 1996; The Expert Panel 1993). Most recommendations restrict consumption of total fat to an upper limit of 30% of daily energy intake.
Low-fat, high-carbohydrate diets are effective in lowering total and LDL cholesterol levels and this has been demonstrated by a number of studies in both men and women (Clevidence et al. 1992; Kasim et al. 1993; Dengel et al. 1995; Kasim-Karakas et al. 1997; Turley et al. 1998; Yu-Poth et al. 1999). These favourable alterations to lipoprotein metabolism by high-carbohydrate diets provided evidence that this type of diet may help CHD prevention. However, these diets have recently come under scrutiny because they also increase plasma triacylglycerol (TAG) and decrease high-density lipoprotein (HDL) cholesterol concentrations (Mensink and Katan 1992).

The observation that high-carbohydrate diets induce fasting hypertriacylglycerolaemia was made as early as in the 1950s (Hatch et al. 1955; Ahrens et al. 1957). Since then, numerous studies have confirmed this “paradoxical” observation (Antonis and Bersohn 1961; Mancini et al. 1973; Jones et al. 1987; Mensink and Katan 1987; Ullmann et al. 1991; Retzlaff et al. 1995; Flynn et al. 1999; Parks et al. 1999). One theoretical argument in favour of the anti-atherogenic utility of low-fat, high-carbohydrate diets has been that postprandial lipaemia will be lower during these diets as the amount of ingested dietary fat is reduced (Connor and Connor 1997). However, studies in healthy men and women and non-insulin dependent diabetes mellitus patients suggest that postprandial lipaemia and diurnal TAG levels are increased, rather than decreased, during the consumption of high-carbohydrate diets compared with diets higher in fat (van Gent et al. 1979; Coulston et al. 1983; Jeppesen et al. 1997; Chen et al. 1993; Chen et al. 1995; Blades and Garg 1995). In addition, controlled-feeding studies (Gonen et al. 1981; Mensink and Katan 1987; Kasim-Karakas et al. 1997; Berglund et al. 1999) as well as intervention studies conducted in freelifing populations (Kasim et al. 1993; Yu-Poth et al. 1999) showed that high-carbohydrate diets also decrease high-density lipoprotein (HDL) cholesterol concentration. These findings suggest that low-fat, high-carbohydrate diets may not reduce overall CHD risk. Accordingly, the wisdom of recommending them as one means for CHD prevention has been an issue of vigorous debate by many reputable scientists (Crouse III 1989; Connor and Connor 1997; Katan et al. 1997; Reaven 1997; Ornish 1998; Rudel 1998; Strain 1998; Connor and Connor 1998; Katan et al. 1998; Grundy 1999).
Fasting TAG (Hokanson and Austin 1996) and HDL-cholesterol (NIH Consensus Conference 1993) concentrations are independent risk factors for CHD. Plasma TAG concentrations show considerable diurnal variation (van Gent et al. 1979). Ingestion of a fat-rich meal results in postprandial elevation of TAG levels lasting for 6 to 8 hours in healthy normolipidaemic individuals. As people in Western Society tend to eat plentiful meals at regular intervals, postprandial lipaemia occurs several times per day, so that most individuals spend ~80% of their lives in the postprandial, rather than the fasted state. From a metabolic point of view, postprandial lipaemia can be considered as a transient or aggravated hypertriacylglycerolaemia and, therefore, augmented and protracted postprandial elevations of triacylglycerol may enhance the development of CHD. Indeed, a number of case-control and descriptive studies have related the magnitude and duration of postprandial lipaemia (Groot et al. 1991; Patsch et al. 1992; Ryu et al. 1992; Nikkilä et al. 1994; Karpe et al. 1998; Karpe et al. 1999; Boquist et al. 1999) and impaired postprandial metabolism of TRL particles (Simons et al. 1987; Karpe et al. 1994; Meyer et al. 1996; Karpe et al. 1999; Rajaratnam et al. 1999) to the presence of CHD and early atherosclerosis. The case-control difference in the postprandial TAG response is very consistent among studies. Usually, cases have higher plasma TAG concentrations in the late postprandial phase or a generally elevated TAG area under the curve even after correction for the fasting level. In addition, the prospective Physician's Heart Health Study demonstrated that raised non-fasting plasma TAG concentration was a strong and independent predictor of future myocardial infarction (Stampfer et al. 1996). Collectively, these findings suggest an association between postprandial TAG metabolism and CHD and, therefore, the augmentation of postprandial lipaemia with high-carbohydrate diets may indicate an atherogenic impairment of lipid profile. Furthermore, since HDL-cholesterol concentration is, at least partly, dependent on the efficiency of postprandial TAG metabolism (Patsch et al. 1983; Patsch et al. 1987), it may be that the reduction in HDL-cholesterol observed as a response to high-carbohydrate diets is a later secondary event associated with the impairment in TAG metabolism.

However, the current anxieties about high-carbohydrate diets may be allayed if ways are found to introduce such diets without adversely affecting plasma TAG metabo-
lism. Physical exercise may be a means of achieving this. Both exercise conditioning 
(Weintraub et al. 1989) and acute bouts of exercise (Aldred et al. 1994; Tsetsonis et 
al. 1997; Gill and Hardman 2000) have been shown to reduce postprandial TAG or 
TRL concentrations, at least when subjects are on their “normal” Western diets. It 
may be, therefore, that exercise, when simultaneously adopted with a high-
carbohydrate diet, could prevent the impairment of TAG metabolism observed with 
this dietary change and, in this way, optimise the effects of high-carbohydrate diets 
on lipoprotein lipid markers with regard to CHD risk. An important issue is that in-
activity is endemic in Western countries, including the UK (Sports Council and 
Health Education Authority 1992), and it is unrealistic to expect previously sedentary 
people to increase their exercise levels dramatically. Moderate exercise is probably 
less daunting than vigorous activity and this is acknowledged in current exercise 
guidelines, which recommend that adults should participate in exercise of moderate 
intensity on most, or preferably all, days of the week (Pate et al. 1995). Thus, with 
regard to public health, what needs to be investigated is the possibility that exercise 
of moderate intensity and duration, can attenuate or negate the hypertriacylglycer-
olaemia observed with a high-carbohydrate diet.

While a small number of studies have looked at the combination of high-
carbohydrate diet and exercise interventions, all of them have studied subjects at the 
fasted state (Liebman et al. 1983; Ullrich and Albrink 1986; Szostak and Cybulksa 
1987; Thompson et al. 1988). To the best of the author’s knowledge there is no in-
formation in the postprandial state. This state is particularly critical and dynamic 
(Miesenböck and Patsch 1992) and could also be a determinant of HDL-cholesterol 
concentration (Patsch et al. 1987; Patsch et al. 1992) and the size of population of 
small dense LDL particles (Karpe et al. 1993b) that are potentially atherogenic 
(Griffin 1999).
AIMS OF THESIS

The main aim of the research presented in this thesis was to investigate whether moderate intensity exercise, when combined with a low-fat, high-carbohydrate, can prevent the impairment of postprandial TAG metabolism which is usually observed with this dietary change. The specific aims of the individual studies were:

Study 1 (Chapter 4)
- To compare the effects of a short-term, low- and high-carbohydrate diet on postprandial TAG responses to a standard test meal.
- To establish a short-term dietary intervention model for study of the hypertriaclylglycerolemic effect of high-carbohydrate diets and thus its prevention.

Study 2 (Chapter 5)
- To test the hypothesis that daily moderate intensity exercise offsets the augmentation of postprandial plasma TAG response associated with a high-carbohydrate diet.

Study 3 (Chapter 6)
- To assess the effects of a high-carbohydrate diet with or without exercise on the TRL particles of exogenous (intestinal) and endogenous (hepatic) origin and their remnants.
CHAPTER 2
REVIEW OF LITERATURE

2.1 Introduction
The aim of this chapter is to describe the scientific background to the studies presented later in this thesis. The first part of the review considers the metabolism of TAG and TRL particles in the postprandial state, its regulation and association with atherosclerosis. The second and third parts deal with the literature regarding the effects of high-carbohydrate diets and exercise, respectively, on TRL metabolism. Potential mechanisms by which TRL metabolism is affected by high-carbohydrate diets and exercise are also discussed.

2.2 Lipids and lipoproteins
Lipids are essential for all living cells and have several important functions: as fuel molecules; as concentrated energy stores; as structural components of cell membranes; and as precursors for steroid hormones. The major lipids circulating in blood are TAG, non-esterified fatty acids (NEFA), phospholipids, cholesterol and cholesterol esters. These lipids differ in their chemical and physical properties. Non-esterified fatty acids, cholesterol and phospholipids are amphiphilic molecules with both hydrophilic and hydrophobic properties, whereas TAG and cholesterol esters are non-polar neutral molecules essentially insoluble in water.

Plasma lipoproteins represent a variety of particles that have the major function of transporting the water-insoluble lipids in the blood. Lipoproteins are particles with a highly hydrophobic lipid core and a relatively hydrophilic outer surface. A typical lipoprotein particle consists of a core of TAG and cholesteryl ester, with an outer surface layer of phospholipid and unesterified cholesterol. Each particle has associated with it one or more protein molecules, the apolipoproteins (apo), which have hydrophobic domains, which "dip into" the core and anchor the protein to the particle, and hydrophilic domains which are exposed at the surface of the particle (Frayn 1996). Lipoproteins consist of a heterogeneous group of particles with different lipid and protein compositions, and different sizes. They have been separated into groups or
fractions on the basis of hydrated density, flotation in an ultracentrifuge or electrophoretic mobility. Today, lipoproteins are most commonly classified according to their hydrated density or Svedberg flotation ($S_r$) value. Svedberg flotation value-index is an operational index calculated from the density ($\mu$), viscosity ($\rho$) of plasma and the total centrifugation force ($g \times $ time) necessary to float a particle to the top by analytical ultracentrifugation (Chen and Reaven 1991). The higher the $S_r$ value, the more buoyant the particle and the greater its TAG content. It should be noted that lipoproteins do not form discrete fractions and each fraction may consist of a range of particles with somewhat different characteristics. The composition and characteristics of the major lipoprotein fractions are presented in Table 2.1. There are four major fractions: Chylomicrons, very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL). This classification also includes chylomicron remnants and intermediate-density lipoproteins (IDL). Chylomicron remnants have similar physical properties to VLDL particles and fall into the VLDL density and flotation rate. IDL particles occupy the 1.006-1.019 g·mL$^-1$ density range and have a $S_r$ value of 12-20. VLDL particles can be subdivided into two groups: VLDL$_1$ (large) with $S_r$ value of 60-400 and VLDL$_2$ (small) with $S_r$ value of 20-60.

Chylomicrons and VLDL particles are relatively rich in TAG and are usually referred to as the TAG-rich lipoproteins (TRL); they are mainly concerned with delivery of TAG from the intestine (chylomicrons) or the liver (VLDL) to tissues. LDL and HDL are smaller particles and are more involved with transport of cholesterol to and from cells (LDL and HDL, respectively).
<table>
<thead>
<tr>
<th>Fraction</th>
<th>Density range (g·ml⁻¹)</th>
<th>Flotation value (Sᵣ)</th>
<th>Diameter (nm)</th>
<th>Major lipids</th>
<th>Major apolipoproteins</th>
<th>Composition (% by weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Protein</td>
</tr>
<tr>
<td>Chylomicrons</td>
<td>&lt; 0.950</td>
<td>&gt; 400</td>
<td>80-1000</td>
<td>Dietary TAG</td>
<td>B48, AI, AII, CI, CII, CIII, E</td>
<td>1</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.950-1.006</td>
<td>20-400</td>
<td>30-80</td>
<td>Endogenous TAG</td>
<td>B100, CI, CII, CIII, E</td>
<td>10</td>
</tr>
<tr>
<td>LDL</td>
<td>1.019-1.063</td>
<td>0-20</td>
<td>20-22</td>
<td>C and CE</td>
<td>B100</td>
<td>20</td>
</tr>
<tr>
<td>HDL</td>
<td>1.063-1.090</td>
<td>0-9</td>
<td>9-15</td>
<td>CE and PL</td>
<td>AI, AII, CI, CII, CIII, E</td>
<td>50</td>
</tr>
</tbody>
</table>

Abbreviations used: Sᵣ, Svedberg flotation index*; C, cholesterol; CE, cholesteryl ester; PL, phospholipid.

* Svedberg flotation index is an operational index calculated from the density (μ), viscosity (ρ) of plasma and the total centrifugation force (g x time) necessary to float a particle to the top by analytical ultracentrifugation. The higher the Sᵣ value, the more buoyant the particle and the greater its TAG content.

Table 2.1. Characteristics of the major lipoprotein classes. Adapted from Frayn (1996) and Mangiapane and Salter (1999).
2.3 Metabolism of postprandial lipid and TRL particles
Most studies on lipid transport have been performed in the postabsorptive state, when TAG metabolism and lipid transport have reached equilibrium. Studies in the postabsorptive state monitor the endogenous pathway of lipid metabolism, whereas studies in the postprandial state monitor the exogenous pathway of lipid metabolism superimposed on that of the endogenous pathway (Kirchmair et al. 1995).

2.3.1 Metabolism of chylomicrons and their remnants: the exogenous pathway
Chylomicrons are secreted by the intestine after fat intake (Green and Glickman 1981, for review) and provide the means to introduce newly absorbed dietary fat into the body. Most dietary lipid (−95 %) is in the form of TAG and under normal conditions absorption of fat is almost complete. The flux of lipid from the gut to the plasma compartment is highly efficient. A meal including about 100 g of fat is easily absorbed in 5 hours (Karpe 1992).

The assembly and secretion of TAG-rich chylomicrons in the intestine are absolutely dependent on the presence of one molecule of apo B48 as the structural protein (Chen and Reaven 1991), which in human subjects is formed exclusively in the intestine after tissue-specific editing of the apo B100 mRNA (Chan 1992). This apo B variant comprises the N-terminal 48 % of the liver-derived variant of apo B, apo B100, and hence its name (Kane et al. 1980). Synthesis of apo B100 has also been described in the human intestine, the quantitative significance of this is however still unknown (Hoeg et al. 1990). It seems that apo B48-containing particles are continuously secreted from the enterocyte and at times of excessive TAG availability (i.e. after fat ingestion) lipid droplets fuse with nascent particles resulting in secretion of very large chylomicrons (Hayashi et al. 1990) containing ~90 % TAG. In addition to apo B48, nascent chylomicrons also contain apo AI, AII and AIV that are de novo synthesised by intestinal cells (Green and Glickman 1981). The chylomicrons secreted by the intestine are transported into the systemic circulation via the lymphatics and enter the blood stream via the thoracic duct in the left subclavian vein. The heart and lungs are the first organs exposed to chylomicrons (Goldberg et al. 2000).
Once the chylomicron has reached the blood stream several compositional changes take place. Apo AI is transferred to circulating HDL particles with reciprocal transfer of apo CI, CII, CIII and apo E. In conditions of low HDL concentrations apo Cs and apo E may be transferred to nascent chylomicrons from resident VLDL particles (Karpe 1999). Apo CII is the essential activator of lipoprotein lipase (LPL) (Cryer 1981), the key-enzyme for the hydrolysis of the massive TAG content of chylomicrons. LPL is located on the surface of capillary endothelial cells of a number of tissues such as heart muscle, lungs, adipose tissue and skeletal muscle (Eckel 1989). The roles of apo CI and apo CIII in the metabolism of TRL are not well understood. Apo CI seems to inhibit the uptake of TRL remnant particles by receptors (Weisgraber et al. 1990) whereas apo CIII seems to inhibit both hydrolysis of TRL-TAG by LPL (Ginsberg et al. 1986) and the removal of TRL by hepatocytes (Windler and Havel 1985). Some TAG is also transferred to LDL and HDL particles in exchange for cholesteryl esters, catalysed by cholesteryl ester transfer protein (CETP) (Miesenböck and Patsch 1992). During the time they spend in the circulation, chylomicrons lose 80 % to 90 % of their TAG content and are subsequently smaller in size (Cohn 1998). During this catabolism, the surface apo As and almost all apo Cs, together with cholesterol and phospholipids, are released and transferred to HDL (Chen and Reaven 1991). The depletion from phospholipids is essential for unmasking of apo E on the surface of the particle, by which the TAG-depleted particle - chylomicron remnant - binds to lipoprotein receptors in liver (Jansen et al. 1998). The chylomicron remnant is rich in cholesteryl ester and contains two major protein components: apo B48 and apo E.

The chylomicron remnant is, under normal conditions, subsequently taken up by specific receptors in the liver. Two receptors appear to have a significant role in the removal process in the liver in vivo: the LDL receptor and the LDL receptor-related protein (LRP) (Beisiegel 1995). For both receptors, apo E is the key ligand mediating the binding and subsequent endocytosis of the particle (Curtiss and Boisvert 2000). The binding of the remnant particle to the LRP may be with direct interaction or, to a much greater extent, by initial binding to heparan sulfate proteoglycans on the hepatocyte surface and subsequent presentation of the particle to the LRP (Beisiegel 1995). Even in the absence of apo E there is hepatic remnant removal which, al-
though it is slow, can clear the particles with some efficiency (Cooper 1997). In the absence of apo E, hepatic lipase may serve as a ligand for the LRP (Cooper 1997; Jansen et al. 1998). LRP could bind chylomicron remnants also through LPL (Beisiegel 1995; Jansen et al. 1998). It has been suggested that, during degradation of chylomicrons by LPL, some LPL remains associated with the chylomicron remnants and is released from its endothelial binding site, subsequently mediating the uptake of remnants by the liver (Jansen et al. 1998). Proteoglycan-bound hepatic lipase on the hepatocyte surface may also serve as a binding site for sequestration, further lipolysis and subsequent direct internalisation of the remnant particle lacking apo E (Cooper 1997; Havel 1998). Alternatively, this particle could be internalised after binding to proteoglycans directly (Cooper 1997; Havel 1998). The last two processes constitute non-receptor mediated uptake of chylomicron remnants by the liver. Once within the hepatocyte, chylomicron remnants are subject to lysosomal degradation, which releases their components for further degradation, storage or resynthesis. Recently, removal of large chylomicron remnants by adipose and muscle tissues has been demonstrated in humans in vivo (Karpe et al. 1997b). This removal may be mediated by the recently discovered VLDL receptor (Wyne et al. 1996; Multhaupt et al. 1996), which recognises apo E as a ligand and is most abundant in skeletal muscle and adipose tissue. The VLDL receptor may be important for delivering of fatty acids from TRL remnants to peripheral tissues, such as muscle and adipose tissue, although this remains to be elucidated.

The half-life of chylomicron-TAG in healthy subjects is only 5-10 minutes (Havel 1994). The capacity of LPL to hydrolyse chylomicron-TAG is very high, so that TAG clearance is not normally saturated even by large fat loads (Havel 1994). This is probably achieved by binding of large chylomicrons to the vascular endothelium i.e. margination (Karpe et al. 1997a; Hultin and Olivecrona 1998). In this way, each particle interacts with several LPL molecules, yielding very rapid catalysis. By contrast, removal of chylomicron remnants by the liver is relatively slow, occurring over 15 minutes or more and remnant clearance is generally saturated after a fat-containing meal (Havel 1997). Thus, the intestine-derived (apo B48-containing) TRL particles found in blood in the postprandial and postabsorptive states can be
considered to be remnants, in the sense that they are at least partially hydrolysed (Havel 1994).

### 2.3.2 Metabolism of VLDL and their remnants: the endogenous pathway

VLDL, which is synthesised in the liver, is the transporter of endogenous TAG in plasma. Hepatic VLDL production is primarily substrate driven, the most important regulatory substrate being fatty acids. Fatty acids may be derived from at least four sources: *de novo* lipogenesis; cytoplasmic TAG stores; fatty acids derived from circulating lipoproteins taken up by the liver; or exogenous (non-esterified) fatty acids (Lewis 1997). The nutritional and hormonal state of the organism determines the rate of VLDL secretion. Therefore, it is the balance between oxidation of fatty acids in the liver and the relative contribution of all of these TAG/fatty acids flux rates, all regulated to a greater or lesser extent by insulin, which ultimately determines the availability of hepatic TAG for VLDL secretion (Karpe 1999). The insulin regulation of VLDL production will be discussed in section 2.4.1.

VLDL particles are secreted continuously from the liver in size and density that ranges across the full $S_r$ 20 to 400 spectrum (Packard and Shepherd 1997). When secreted they contain, as well as TAG (the main lipid), a single molecule of apo B100 together with other apolipoproteins, including apo A1, AII, AIV, the three apo Cs and apo E (Havel 1997). Newly secreted VLDL particles acquire additional apo E and C from circulating lipoproteins, mainly HDL. The catabolism of VLDL is similar to that of chylomicrons, with the TAG undergoing hydrolysis by LPL (facilitated by apo CII) and the redundant surface material being passed to other particles, mainly HDL. Exchange of lipoprotein core lipids between cholesterol-rich particles (LDL and HDL) and VLDL is catalysed by CETP. VLDL remnants are produced at a considerable slower rate than chylomicron remnants and, therefore, VLDL may circulate normally without appreciable hydrolysis for several minutes or more (Havel 1997). As the VLDL particle becomes more delipidated, the remnants can either be taken up by the liver or they can continue through a "cascade" of delipidation to form IDL and finally LDL. The ultimate remnant of VLDL is LDL.
There is evidence to suggest that the majority of the largest, VLDL₁ particles, after hydrolysis are cleared directly from plasma and only a minor fraction (~10 %) appears in the LDL pool (Packard et al. 1984). There is even evidence that an appreciable proportion of VLDL₁ is directly removed from the circulation without appearing in the VLDL₂ fraction (Packard and Shepherd 1997). On the contrary, the smaller, VLDL₂ particles which contain products of VLDL₁ delipidation and newly-secreted VLDL particles, are rapidly and substantially (> 40 %) converted to LDL, suggesting that the major precursor of the latter is to be found in the VLDL₂ subfraction (Packard et al. 1984). Interestingly, substantially metabolic heterogeneity is observed even within the VLDL₂ fraction. VLDL₂ particles that are derived by catabolism of VLDL₁ are catabolised slowly within their flotation interval without being significantly transferred to LDL. Conversely, directly synthesised VLDL₂ are rapidly converted into LDL (Packard et al. 1984). Similar processes to those for chylomicron remnants are likely to mediate the clearance of VLDL₁ and VLDL₂, although the LDL receptor pathway seems to be the most important in the catabolism of VLDL₂ (Packard and Shepherd 1997).

IDL are only present in low concentration in normal human plasma as it represents an intermediate stage in the formation of LDL from VLDL. It has a low TAG and a high cholesteryl ester content than the VLDL and retains the apo B100 and apo E. IDL can either be uptaken by liver or become LDL particle through the action of LPL and mainly hepatic lipase and with loss of apo E. LDL consists of a cholesteryl-ester enriched core with a shell containing phospholipid, free cholesterol and apo B100 (Frayn 1996). It is removed from the circulation after binding to LDL receptors either in the liver (80 %) or in extrahepatic tissues 20 % (Mangiapanne and Salter 1999). The half-life of LDL is several days.

2.3.3 Integration of TRL metabolism in the postprandial state
In the postprandial state, chylomicrons and VLDL mix in blood and the two TRL species compete for the same lipolytic pathway, the action of LPL (Brunzell et al. 1973; Karpe and Hultin 1995; Björkegren et al. 1996). During this period, there is an increase in the concentration of TAG and TRL particles in plasma but the contribution of each TRL species to these increases is largely different. The postprandial in-
crease in plasma TAG concentration seems to be predominantly (~80 %) due to TAG contained within apo B48-containing TRL particles (Cohn et al. 1993). Conversely, the increase in apo B100-containing TRL observed postprandially (Cohn et al. 1988a; Karpe et al. 1993a; Schneeman et al. 1993) can account for as much as 80 % of the postprandial increase in the total apo B-containing TRL particles (Schneeman et al. 1993). Thus, postprandial lipaemia predominantly represents the presence in plasma of large TRL from the intestine (chylomicrons), but also reflects the plasma accumulation of TRL particles of hepatic origin. It has been calculated that endogenous (apo B100) TRL constitute 96-97 % and 91-96 % of all TRL particles in the fasted and postprandial state, respectively (Karpe 1999). Hence, the number of exogenous TRL particles appearing postprandially is small and the basal production rate and turnover of endogenous TRL are important factors determining the total lipoproteinaemic rise after fat intake.

The postprandial accumulation of apo B100 TRL particles has been a subject of research and two main explanations have been given for this phenomenon: i) reduced clearance and/or ii) increased hepatic secretion. The rationale behind the first explanation is that chylomicrons and VLDL compete postprandially for a common removal pathway through LPL (Brunzell et al. 1973), which seems to hydrolyse chylomicron-TAG in preference to VLDL-TAG (Ports et al. 1991). Therefore, in the postprandial state chylomicrons may be preferentially hydrolysed leading to accumulation of VLDL particles. A number of studies have supported this speculation (Schneeman et al. 1993; Karpe et al. 1993a; Karpe and Hultin 1995; Björkegren et al. 1996). Schneeman et al. (1993) observed a remarkably close correlation between the average concentration, as well as the increment in concentration of TRL apo B48 and apo B100 postprandially. They concluded that these results were consistent with impairment of hydrolysis of VLDL-TAG as a result of preferential hydrolysis of chylomicron-TAG by LPL, leading to VLDL accumulation. This line of reasoning was also followed by Karpe et al. (1993a) who presented the important finding that postprandial increases in the concentration of apo B48 TRL was accompanied by elevation of VLDL$_1$ and reduced concentration of VLDL$_2$ particles. Additionally, the peak plasma level of the VLDL$_1$ seemed to coincide with that for large chylomicron remnants, arguing for an impeded degradation of VLDL particles. Karpe and Hultin
(1995) showed that endogenous TRL accumulate in rat plasma due to a failure to compete with a chylomicron-like TAG emulsion for a common lipolytic pathway. This contention was subsequently supported in humans by Björkegren et al. (1996) who, using a short-term intravenous infusion and stable isotope techniques, demonstrated that the emulsion caused a 75-90% block of the conversion of VLDL₁ to VLDL₂ and there was no sign of enhanced synthesis of VLDL₁ after infusion of the TAG-emulsion. The implication of this finding is that VLDL₁ lipolysis does not "compete" effectively with chylomicron clearance and so is virtually suspended when the plasma content of chylomicrons is high, resuming when lipid absorption is complete.

It has been suggested that substrate delivery to the liver is the major determinant for VLDL secretion (Sniderman and Cianflone 1993). Therefore, hypothetically an increase in postprandial hepatic secretion of VLDL particles might occur because of increased substrate availability, for example fatty acids derived from partially hydrolysed lipoproteins taken up by the liver, or lipids reaching the liver via the portal vein. Furthermore, LPL-derived fatty acids that are not efficiently entrapped by the adipose tissue (Frayn et al. 1997) may also serve as a substrate for VLDL secretion. In line with this reasoning, Cohn et al. (1990) studied the effect of fasted and fed conditions on the synthetic rate of VLDL apo B₁₀₀ using a stable isotope (deuterated leucine) dilution technique. They reported that secretion of apo VLDL apo B₁₀₀ is increased by ~50% in the fed state over that observed in the postabsorptive state. However, it was also reported that the rate of secretion of apo B₁₀₀ in VLDL was ~50% of the rate of production of LDL apo B₁₀₀ in the fasted state, which raises some question about the method used (Havel 1994). Specifically, it has been reported that a patient homozygous for LPL deficiency who was heterozygous for familiar hypercholesterolaemia had very low LDL-cholesterol levels (Zambon et al. 1993), a finding inconsistent with appreciable direct secretion of LDL particles from the liver. In summary, the majority of the evidence indicates that the postprandial accumulation of VLDL particles is due to competition with chylomicrons for hydrolysis of TAG by LPL rather than increased VLDL secretion from the liver.
2.4 Regulation of postprandial TAG metabolism

Postprandial TAG metabolism is regulated by a number of factors. The hormone insulin and the enzyme LPL are discussed in this section.

2.4.1 Insulin

Insulin is an important regulator not only of carbohydrate but also of TAG metabolism as well. In the postprandial state, insulin regulates both VLDL production and TRL-TAG hydrolysis by LPL (Frayn 1993). *In vivo* evidence suggests that acute hyperinsulinaemia suppresses VLDL-apo B and VLDL-TAG production by about 50% in humans (Lewis *et al.* 1993; Lewis *et al.* 1995). This effect seems to be due to a number of mechanisms. First, insulin decreases NEFA availability for VLDL-TAG synthesis by decreasing intracellular hormone-sensitive lipase (HSL) activity in adipose tissue (Coppack *et al.* 1994; Frayn *et al.* 1994) by dephosphorylation. Second, it appears that insulin (maybe in combination with the acylation-stimulating protein (Sniderman *et al.* 1997; Sniderman *et al.* 2000)) exerts a direct effect on the esterification of fatty acids in adipose tissue and thus maintenance of a concentration gradient for the flow of fatty acids from the site of LPL action to the adipocytes (Frayn *et al.* 1994). This effect is particularly important in the postprandial state when a major proportion of LPL-derived fatty acids may escape retention into the adipose tissue, accumulate in the capillary microenvironment and finally enter the plasma NEFA pool (Frayn *et al.* 1997). These fatty acids may have a negative effect on LPL activity (Eckel 1989), impairing TRL-TAG hydrolysis, and after entering the NEFA pool, may serve as an excellent substrate for VLDL-TAG synthesis. Insulin action, therefore, is to enhance entrapment of LPL-derived fatty acids into adipose tissue by stimulating their reesterification. Finally, insulin suppresses VLDL-TAG production, even in the presence of elevated NEFA, indicating that its suppressive effect is partly due to an NEFA-independent process (Lewis *et al.* 1995). A recent study (Malmström *et al.* 1997) demonstrated that VLDL secretion is under complex regulation as insulin acutely suppresses VLDL₁-apo B production but has no effect on VLDL₂-apo B production from the liver. VLDL₁ and VLDL₂ production is, therefore, independently regulated. In the postprandial state this makes sense in that, with an ample supply of chylomicron-TAG for muscle and adipose tissue to utilise, there is little need for the liver to secrete large TRL particles.
Insulin also directly stimulates LPL in adipose tissue (Eckel 1989), which is a major site of TRL-TAG hydrolysis. The stimulation of adipose tissue LPL by insulin is a complex and not well defined process and seems to involve increased transcription as well as post-transcriptional (probably changes in LPL mRNA stability) and post-translational mechanisms (Braun and Severson 1992). Elucidation of the precise biochemical mechanism is difficult as in vitro studies the cellular preparation may matter. A study in diabetic subjects treated with insulin (Simsolo et al. 1992) showed that adipose LPL activity increased without change in mRNA levels, suggesting post-transcriptional events. In skeletal muscle of healthy subjects, insulin has a suppressive effect on LPL activity (Kiens et al. 1989; Yost et al. 1995). The tissue specific regulation of LPL by insulin has been clearly demonstrated by Farese et al. (1991). These authors showed that, in normal-weight subjects, insulin infusion, under conditions of euglycaemia, has a stimulatory effect on adipose LPL but an inhibitory effect on skeletal muscle LPL activity. However, these findings should be extrapolated to the more realistic situation of the postprandial state with caution as it has recently been reported (Yost et al. 1998) that both skeletal and adipose tissue LPL activity (assessed in biopsy tissue samples) increase significantly from fasted to post-prandial state.

2.4.2 Lipoprotein lipase
LPL is synthesised in parenchymal cells of several tissues. Under processing in the endoplasmic reticulum and Golgi complex is translocated, by presently unknown mechanisms, to functional heparan sulphate proteoglycan binding sites on the luminal surface of the capillary endothelium, where the hydrolysis of TRL particles takes place (Braun and Severson 1992). The LPL activity of the white adipose tissue and muscular tissues (skeletal muscle and cardiac muscle) accounts for a high proportion of the total activity of the body and these tissues are most important, therefore, in the bulk removal of TAG from plasma (Cryer 1981). LPL is considered as the rate-limiting step for plasma TRL-TAG hydrolysis. However, it is important to appreciate that several factors other than LPL are also critical modulators of this process including the apolipoprotein composition of the particles, the size of the lipoproteins and how LPL is displayed along the endothelial luminal surface (Goldberg 1996).
LPL activity can be altered in a tissue-specific manner, which is physiologically important, because it directs fatty acid utilisation according to the metabolic demands of individual tissues. For example, fasting results in a reduction in LPL activity in adipose tissue but stable or increased levels of LPL in muscle whereas feeding has exactly the opposite effects, at least in the adipose tissue (Eckel 1989). These effects are probably orchestrated by insulin that is normally secreted after consumption of a mixed meal. Adipose tissue LPL action (measured as TAG extraction across subcutaneous adipose tissue) is low (but not completely inactive) in the fasted state and rises postprandially to final values two to three times those in the fasted state (Frayn et al. 1994; Fielding and Frayn 1998). Adipose tissue LPL action reaches its peak at four to five hours postprandially (around three to four hours of insulin stimulation), which coincides with the peak levels of TAG in plasma (Coppack et al. 1992; Frayn et al. 1994). Taking into consideration that adipose tissue appears to have a predominant role in postprandial clearance of circulating TAG, at least in sedentary subjects (Coppack et al. 1990, Potts et al. 1991), this represents a remarkable way in which TAG metabolism is regulated. However, as already mentioned in section 2.4.1, muscle LPL activity probably also increases postprandially (Yost et al. 1998) and, therefore, muscle tissue contributes to TAG hydrolysis after the consumption of a meal.

2.5 Factors affecting postprandial lipaemia
Several factors, of which the postabsorptive plasma TAG concentration, preceding diet, age, gender and exercise seem to be the most important, affect postprandial TAG metabolism. These factors are discussed in this section except exercise, which will be discussed separately in section 2.9.2.

2.5.1 Postabsorptive plasma TAG concentration
Early studies by Denborough (1963) and Nestel (1964) demonstrated, in both CHD and control subjects, a close relationship between the degree of postprandial lipaemia and fasting TAG levels. Indeed, the endogenous TAG pool, which mainly represents VLDL particles, is an important determinant of the evolution of TAG concentration after fat intake (O’Meara et al. 1992; Potts et al. 1994). Since chylomicrons and VLDL compete for the same lipolytic pathway (Brunzell et al. 1973; Björkegren et al. 1996), an increase in the fasting VLDL concentration leads to competition and
subsequent delay in the hydrolysis of TRL particles postprandially, affecting the magnitude of postprandial lipaemia. Thus, when evaluating the postprandial response, it is essential to consider the basal levels of TAG and TRL particles. Experimental designs in which subject groups have comparable TAG and TRL levels could permit more robust conclusions that those where data have been adjusted to correct for differences in the basal state.

Despite the close relationship between fasting and postprandial TAG levels, postprandial TAG response is often independent of basal TAG levels. This has been well demonstrated in studies where subjects exhibited higher postprandial lipaemia than others with similar fasting TAG concentration (Cohen et al. 1989; Merrill et al. 1989). More importantly, postprandial TAG and TRL levels appear to be discriminators for the presence of CHD, independently of baseline TAG levels. CHD patients with normal fasting TAG concentration have been shown to exhibit higher postprandial TAG (Groot et al. 1991) and TRL apo B48 (Rajaratnam et al. 1999) levels than controls matched for baseline TAG concentration. The most convincing evidence of an association between exaggerated postprandial lipaemia and CHD risk, in the absence of elevated fasting plasma TAG, was provided by a study of (Uiterwaal et al. 1994). The postprandial responses of sons of CHD patients were compared with those of healthy men. Although there was no difference in postabsorptive plasma TAG levels between groups, postprandial TAG concentrations at late time points were significantly higher in the sons of men with CHD. Therefore, postprandial TAG concentrations may be more closely related to atherogenic risk than those observed in the postabsorptive state.

2.5.2 Preceding diet
Altering the macronutrient composition of the background diet substantially influences postprandial TAG metabolism. Despite they contain less fat, low-fat, high-carbohydrate diets exaggerate postprandial lipaemia. The effects of high-carbohydrate diets will be discussed in detail in section 2.8.2. A relationship also exists between postprandial TAG response and saturation of dietary fatty acids. Supplementation of long-chain ω-3 polyunsaturated fatty acids leads to decreases in both fasting and postprandial TAG concentrations, even in doses as low as 1 g per day.
(Roche and Gibney 2000). Dietary studies in human volunteers generally support the view that diets high in polyunsaturated fatty acid, particularly of ω-3 series, result in reduced fasting and postprandial TAG concentration and decreased residence time in the circulation of both chylomicron remnants and VLDL particles as compared to diets rich in saturated fat (Bergeron and Havel (1997) and Williams (1997) for review). These effects are evident in postprandial responses to both polyunsaturated fat-rich and saturated fat-rich meals. Diets high in monounsaturated fatty acids seem to affect the pattern rather than the magnitude of the response compared to diets enriched in saturated fat. A recent cross-cultural study (Zampelas et al. 1998) comparing Northern and Southern Europeans (habituated to saturated fat-enriched and monounsaturated fat-enriched diets, respectively) showed that subjects from Southern Europe exhibited a pronounced early increase in plasma TAG levels in response to fat meal, with a rapid return to fasting concentrations. In contrast, Northern Europeans had a slow rise in TAG levels with sustained elevation beyond the six-hour postprandial period. The pattern of the postprandial TAG response in the two groups was not influenced by the test meal fatty acid composition (saturated fat-rich and monounsaturated fat-rich meal). These findings were subsequently confirmed by Roche et al. (1998) who conducted a study in which subjects were given a standard olive oil test meal twice: at the end of an eight-week monounsaturated fat-enriched diet and after an eight-week saturated fat-enriched diet. After subjects had consumed the monounsaturated fat-rich diet peak plasma TAG and TRL apo B48 concentrations occurred earlier in the postprandial phase.

2.5.3 Age and gender
There is evidence that increasing age is accompanied by a greater postprandial response. In a study by Cohn et al. (1988b), younger subjects (29 ± 2 years) exhibited a response that was 40% lower than that of older subjects (66 ± 2 years). However, the older subjects also had higher fasting plasma TAG that, as already discussed, may have determined the difference in the postprandial response. The same study also showed that lipaemia is lower in females than males at all age groups. Tollin et al. (1985) reported that TAG clearance was greater in women than men for all age groups, although TAG clearance rates decreased with age for the females but not the males.
2.6 Methods for studying TAG and TRL metabolism

The TAG metabolic capacity can be examined by a fat challenge, which is usually administered orally or intravenously. Presently, there is no consensus of how a standardised postprandial state should be elicited (for example dose or contents of an experimental meal), which makes comparisons of results of studies difficult.

2.6.1 Oral fat tolerance test

The oral fat tolerance test is an easy way to assess TAG metabolic capacity and which mimics the physiological situation. Postprandial lipaemia can be quantified as the total area under TAG concentration vs time curve (AUC) or as the incremental area, which is the total area normalised for the baseline values (Patsch 1987). The size and composition of the fat meal utilised vary considerably. The size of the test meal can be adjusted according to body surface area, lean body mass, body mass or can be the same for all subjects. In a study in healthy young men (Cohen et al. 1988), subjects were fed liquid formulas containing 40 g, 80 g and 120 g of cream fat and postprandial TAG levels were measured over 8 hours. The lipaemic response (8-hour AUC) was proportional to the amount of fat ingested, which may indicate that removal of TAG was not saturated by any of these amounts. Dairy cream is widely used despite some possible disadvantages (Karpe 1992). Dairy cream contains medium-chain fatty acids that are not re-esterified in the enterocyte but are transported directly to the liver via the portal vein. This might obscure the postprandial plasma TAG response. Additionally, dairy cream may vary in fatty acid composition depending on the season. In both these respects, common vegetable oils like corn oil or olive oil seem to be suitable although less palatable than dairy cream. Soybean oil has also been used by some researchers (Cohn et al. 1988a; Karpe et al. 1993a). Cohn et al. (1988a) investigated postprandial TAG and TRL responses in subjects who consumed fat as soybean oil or as soybean oil plus cream and found no differences.

Both carbohydrate and protein should be supplied together with the fat to resemble the physiological situation, especially by eliciting a postprandial insulin response. Interestingly, simultaneous ingestion of carbohydrate and fat affects postprandial lipaemia. Glucose ingestion along with a fat load has been shown to diminish (Cohen
and Berger 1990) or not to affect postprandial lipaemia (Cohen and Schall 1988). In contrast, fructose and fructose-containing carbohydrates (e.g. sucrose) augment the postprandial lipaemia induced by a fat meal (Cohen and Schall 1988; Jeppesen et al. 1995). The composition of the fat in the meal also has an effect on postprandial lipaemia. There is evidence of differences in postprandial lipaemic responses to meals containing predominantly saturated, monounsaturated, ω-6 polyunsaturated and ω-3 polyunsaturated fatty acids, with levels of TAG response in the order ω-3 polyunsaturated fatty acids < ω-6 polyunsaturated fatty acids < monounsaturated fatty acids < saturated fatty acids (Williams 1997). Since the composition of the test meal affects postprandial lipaemia, it is necessary that an identical test meal is used when comparing postprandial TAG responses after different interventions, in order to be able to isolate the effects of the interventions from the acute effects of the test meals.

2.6.2 Intravenous fat tolerance test
With this methodology an artificial TAG emulsion (e.g. Intralipid) is injected intravenously and venous blood samples are obtained shortly at various intervals (e.g. 5-minute intervals for 40 minutes). This test provides a measure of TAG clearance, independent of absorption processes in the gastrointestinal tract and the rate of synthesis and influx of chylomicrons into the circulation. Elimination follows first-order kinetics (i.e. the clearance rate is directly proportional to the concentration) with a constant fraction of the emulsion cleared from the plasma per unit time (Kirchmair et al. 1995). The calculated slope is a measure of the TAG-clearance capacity. This method assumes that VLDL production and degradation is constant for the duration of the test and that the particles in the TAG emulsion behave as chylomicrons.

2.6.3 Oral retinyl ester assay
The aim of this test is to distinguish between exogenous and endogenous TRL particles. The presence of intestinal TRL can be detected by measuring plasma retinyl esters (Kirchmair et al. 1995). The rationale for this approach is based on the concept that orally ingested vitamin A is esterified in the intestine and is incorporated into the core of chylomicron particles. These lipoproteins are secreted into intestinal lymph, and their component TAG is hydrolysed by LPL in peripheral tissues. Retinyl esters
are believed to remain associated with chylomicrons during lipolysis, not to be exchanged between lipoproteins in plasma and to be taken up by the liver within chylomicron remnant particles. Evidence suggests that the liver does not resecrete these retinyl esters and that they are either stored in liver or resecreted as unesterified retinol bound to retinol binding protein. Therefore, they have been used as markers in plasma for lipoproteins of intestinal origin. It is clear that in the hours immediately after vitamin A and fat ingestion, the majority of plasma retinyl esters are indeed associated with apo B48-containing TRL particles (Cohn et al. 1993). However, later after a meal, a significant proportion of plasma retinyl esters is associated with apo B100 lipoproteins within the TRL as well as the LDL fraction (Krasinski et al. 1990). Furthermore, the retinyl palmitate does not provide uniform labeling of chylomicrons as it is probable that the larger species carry more retinyl palmitate than the smaller ones (Karpe et al. 1995).

2.6.4 Direct measurement of apo B48 and apo B100 in the TRL fraction

This is the most accurate way for quantification of TRL particles of exogenous and endogenous origin, although it is a major methodological challenge. The determination of apo B48 and apo B100 in TRL fraction is difficult for several reasons (Karpe and Hamsten 1995). First, the amino acid sequence of apo B48 is identical to the N-terminal 48% of apo B100. Second, apo B48 is present at very low concentrations in plasma compared with apo B100. Third, the expression of apo B epitopes may vary depending on the lipid content of the particle. These circumstances restrict the basis for using immunochemical techniques to quantify the two apo B species accurately. Thus, attempts to measure TRL apo B48 and apo B100 concentrations have focused on analytical sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE). A method by Karpe and Hamsten (1994) allows the sensitive quantification of TRL apo B48 and apo B100 concentrations in plasma, without differences in chromogenicity between apo B48 and apo B100, by applying restricted masses of apo B on the gel. In addition, plasma and TRL-apo B48 concentrations have been determined using a competitive enzyme-linked immunosorbent assay (ELISA) (Lovegrove et al. 1996). With this method, a specific polyclonal anti apo B48 antiserum is used, which recognises the C-terminal region of the protein on the surface of lipoprotein particles, showing no cross-reaction with apo B100 (Peel et al. 1993).
2.7 Postprandial TAG metabolism and atherosclerosis

Relationships between plasma TAG concentration and CHD have largely been described in the fasted state (Hokanson and Austin 1996). Triacylglycerol itself is not a component of plaques and there is a debate as to whether the effects of TRL particles on atherosclerosis are direct or indirect (Grundy and Vega 1992). Postprandial lipaemia is transient hypertriacylglycerolaemia, which affects the physical characteristics and particle composition of all major plasma lipoproteins in a way that it may contribute to the initiation and progression of atherosclerosis and CHD. In the past decade, there is a wealth of evidence for a potential link between postprandial TAG metabolism and the development of atherosclerosis.

An independent association between increased postprandial TAG levels and CHD has been demonstrated by Patsch et al. (1992) in a study investigating 61 male normolipidaemic subjects with severe CHD and 40 control subjects as verified by angiography. Patients with CHD exhibited a higher and prolonged postprandial TAG response compared to controls. By multivariate logistic-regression analysis, including HDL cholesterol, postprandial TAG concentrations were independently associated with the disease, whereas fasting TAG level was not. Notably, postprandial TAG concentration (6 hours) predicted the presence or absence of CHD with accuracy (68 %) which was higher than that of HDL2-cholesterol (64 %). In another study, postprandial TAG response was again pronounced and late in normolipidaemic patients with severe coronary atherosclerosis compared to matched controls (Groot et al. 1991). Similar results have also been reported by others (Nikkilä et al. 1994).

Postprandial TAG concentrations seem to be a potential discriminator not only for the presence of severe CHD but for early atherosclerosis as well. In healthy asymptomatic subjects, a commonly used surrogate marker for early atherosclerosis is the intima-media thickness of the common carotid artery, measured by B-mode ultrasound. Ryu et al. (1992) reported a strong and independent association between the peak postprandial TAG concentration and the carotid artery intima-media thickness in 47 healthy middle-aged men and women. In accordance with these results, postprandial TAG response (expressed as incremental AUC) in asymptomatic mildly hyperlipidaemic individuals (Sharrett et al. 1995) and late (6 hour) postprandial TAG
concentration in healthy men (Karpe et al. 1998) have been positively associated with early atherosclerosis, independently of both fasting TAG and LDL cholesterol levels. Boquist et al. (1999) recently demonstrated an independent association between early (2 hour), rather than late, postprandial TAG levels and carotid artery intima-media thickness in healthy men. A possible explanation for this rather "unusual" finding given by the experimenters was the inclusion of apo E3 homozygotes only, because postprandial lipoprotein metabolism is impaired in individuals carrying the apo E4 allele (Bergeron and Havel 1996). Notably, in the majority of the above mentioned studies (Ryu et al. 1992; Karpe et al. 1998; Boquist et al. 1999), HDL-cholesterol did not correlate significantly with early atherosclerosis even in univariate analyses, implying that this lipid may not be a discriminator of the early phase of CHD.

The precise way in which postprandial TAG metabolism affects the pathogenesis and progression of CHD has not been elucidated yet but it seems that it is associated with a constellation of potentially atherogenic lipoprotein changes. These include: i) increase in the plasma concentration of TRL particles and their remnants; ii) decrease in HDL-cholesterol due to core lipid exchange between HDL and postprandial TRL; and iii) formation of small dense LDL that are potentially atherogenic.

2.7.1 Postprandial increase in TRL particles and their remnants
Zilversmit (1979) was the first to propose a link between postprandial TRL remnants (principally chylomicron remnants) and atherosclerosis. He proposed that atherogenesis involved the binding of these remnants to the arterial wall, hydrolysis of their TAG by arterial LPL and subsequent internalisation of the remnant by arterial smooth muscle cells. Four case-control studies suggest a relationship between elevated postprandial levels of apo B48-containing TRL and the presence of CHD. Two of them were performed in women (Meyer et al. 1996; Rajaratnam et al. 1999) and two in men (Simons et al. 1987; Karpe et al. 1994). The study by Meyer et al. (1996) showed that fasting, but not postprandial, apo B48-containing TRL particles were higher in women who had developed CHD in the presence of normal LDL- and HDL-cholesterol concentrations, but increased non-HDL-cholesterol, compared with matched controls. The authors speculated that this was probably due to increased se-
cretion of these particles by the intestine in the fasted state. In the study by (Rajaratnam et al. 1999), the authors compared postprandial metabolism of TAG and apo B48-containing TRL particles between normolipidaemic postmenopausal women with CHD and controls. The two groups were matched for age and body mass index and had similar fasting plasma and lipoprotein concentrations of major lipids (except lower LDL-cholesterol) and apo E phenotype distribution. There was no difference in the plasma postprandial TAG response between cases and controls. However, the cases had higher fasting TRL apo B48 levels and incremental AUC than the controls even after adjustment for baseline TRL apo B48 and LDL-cholesterol concentrations. The authors inferred that the clearance of intestinally derived lipoproteins is diminished in women with CHD. Unfortunately, in both these studies the apo B100-containing TRL particles were not measured.

Simons et al. (1987) demonstrated a higher apo B48 to apo B100 ratio in the S_f > 60 fraction at 4 hours postprandial in CHD male patients compared to controls. However, in this particular comparison, the controls had significantly lower fasting TAG concentration, which could have been reflected in their lower apo B48 to apo B100 ratio at 4 hours (the time of maximal postprandial lipaemia). When the same comparison was made with a baseline TAG-matched control group, no difference was observed between cases and controls. In a comprehensive study by Karpe et al. (1994), small chylomicron remnants (apo B48-containing TRL in the S_f > 20-60 fraction) correlated positively with the rate of progression of coronary artery atherosclerosis, as assessed by coronary angiographies separated by approximately five years in 32 male patients with CHD. This relationship was independent of HDL-cholesterol and small dense LDL particle levels. Finally, one study which has investigated cases with early atherosclerosis, rather than CHD, demonstrated a higher TRL apo B48 to apo B100 ratio at 8 hours postprandially in cases than in controls who were not, however, matched for baseline lipids and other risk factors for CHD (Sharrett et al. 1995).

Although a number of studies have examined the potential involvement of intestinally derived TRL particles in atherosclerosis, as originally suggested by Zilversmit, closer examination of lipid metabolism and the interactions between lipoproteins that
occur in the postprandial state has directed the interest to apo B100-containing TRL particles as well. A recent study in humans (Karpe et al. 1997a) showed that a major proportion of chylomicron remnants are removed from plasma long before they attain a size at which they may penetrate the arterial wall. Furthermore, recent detailed examinations of compositional features of human fasting and postprandial chylomicron and VLDL particles (Björkegren et al. 1997; Björkegren et al. 1998) have revealed that VLDL particles attain remnant characteristics in the postprandial state. Specifically, both large and small VLDL become cholesterol, apo E- and apo CI enriched, but depleted in apo CII in the postprandial state. Large and small chylomicron remnants contain significantly more apo CII molecules per particle than VLDL in fasted and postprandial states, which helps to explain the reduced efficiency of VLDL, and especially VLDL₁, for lipolysis postprandially. The transient increase (by 50 %) in cholesterol content (Björkegren et al. 1997) and concentration (Karpe et al. 1993a) of VLDL₁ in the postprandial state could explain why the postprandial TRL accumulation of cholesterol is mainly accounted for by VLDL particles (Schneeman et al. 1993). Apo E-enriched VLDL₁ particles can be uptaken by macrophages in the arterial wall through rapid, receptor-mediated processes and, therefore, contribute at least to the initiation of foam cells in the arterial wall (Bradley and Gianturco 1994). Notably, VLDL₁ carry three to six times more cholesterol than one LDL particle (Bradley and Gianturco 1994). This may suggest a role for postprandial VLDL in atherogenesis. An important contribution to this issue has been made by Rapp et al. (1994) who used immunoaffinity chromatography to isolate TRL particles from human atherosclerotic plaque removed during surgery. They found about one-third of the lipoprotein-associated cholesterol in the IDL/VLDL fraction. Plaque IDL and VLDL were significantly larger than the respective equivalents from plasma and were substantially apo E-enriched, i.e. they had remnant characteristics. Apo B48 was not found. Indirect evidence for a link between VLDL and CHD is also given by Karpe et al. (1999) who recently compared the pattern of TRL particles in the postprandial state in hypertriacylglycerolaemic subjects with and without manifest coronary atherosclerosis. The postprandial accumulation of large VLDL was the most pronounced discriminator between these groups whereas there was no disproportionate increase in apo B48-containing particles.
At the moment, there is no evidence that the type of apo B itself has an effect on the extent of atherosclerosis. A recent study (Véniart et al. 1997) examined the extent of atherosclerosis in apo E knockout mice that synthesise exclusively either apo B48 or apo B100. The type of apo B did not have an effect on susceptibility to atherosclerosis. In conclusion, it seems that the atherogenic potential of a lipoprotein species depends on its actual concentration in plasma, size and ability to interact with macrophage scavenger receptors. All the current data suggest that perturbed postprandial TRL metabolism is related to atherosclerosis. However, there is currently limited evidence for an association between CHD and a specific accumulation of chylomicron remnants compared to apo B100 TRL remnants.

As the metabolism of exogenous and endogenous TRL particles are so tightly linked and remnants of both origins have been implicated in atherosclerosis, it is reasonable to discuss studies relating TRL remnants and CHD. Remnant lipoproteins are hard to define and even harder to isolate. They are the products of partial delipidation of TRL particles. As such they can exist throughout the Sf 0-400 density range alongside new secreted particles. Remnants are cholesteryl-ester rich and have a high content of apo E and little apo CII. There is a wealth of evidence linking TRL remnant particles and risk or progression of atherosclerosis (Hodis and Mack 1998; Hodis 1999). Small cholesteryl-ester rich VLDL particles have been related to CHD severity in young hypertriacylglycerolaemic postinfarction patients (Tornvall et al. 1993). In vivo measurements of fluxes of various lipoprotein species across the intima of human carotid arteries have also shown that elevated plasma concentrations of remnant lipoproteins (Sf 12-60) shared with LDL the potential for causing lipid accumulation in the arterial intima (Shaikh et al. 1991). Recently, a novel immunoaffinity method (Nakajima et al. 1993) has been developed to isolate remnant-like lipoprotein particles (RLP) and measure their cholesterol content (section 3.11.6). Increased levels of RLP-cholesterol have been found in normolipidaemic men with CHD (Devaraj et al. 1998). RLP-cholesterol concentration has also been more strongly related with the severity score of coronary atherosclerosis in cases of sudden cardiac death than LDL-cholesterol concentration (Takeichi et al. 1999). Recently reference values have been published from the Framingham Study (McNamara et al. 1998) but quantification of RLP-cholesterol in hard endpoint studies is still lacking.
2.7.2 Low HDL-cholesterol

HDL particles are the smallest and densest of the lipoprotein classes. HDL regulates the removal and transfer of excess cholesterol from peripheral tissues to the liver, i.e. reverse cholesterol transport. According to hydrated density using preparative ultracentrifugation, HDL can be separated into mainly two subclasses, the lighter and larger HDL₂ and the denser and smaller HDL₃. HDL₂ contains three to four times more cholesteryl ester and TAG molecules than HDL₃ and is twice as "efficient" as HDL₃ as a vehicle for fat transport (Eisenberg 1984).

HDL and TRL metabolism is tightly linked so that constituents (free cholesterol and phospholipids) originating from the surface coat of lipolysed TRL particles constitute the major, if not the only, source of HDL lipids (Eisenberg 1984). Fasting HDL-cholesterol concentration shows a strong inverse association with plasma TAG levels in the fasted state (O'Meara et al. 1992) and postprandially (Patsch et al. 1983). It is likely that hypertriacylglycerolaemia, either as a metabolic disorder or a transient postprandial phenomenon, results in accelerated exchange of HDL-cholesteryl ester for TRL-TAG mediated by CETP. The TAG-enriched HDL particle is eventually remodeled by the action of hepatic lipase, which hydrolyses its TAG and phospholipid, leading to the formation of smaller HDL. This remodeling promotes HDL catabolism, lowering the concentration of the cardioprotective and metabolically active HDL₂ fraction and thus impairing reverse cholesterol transport (Tall 1992). A recent kinetic study (Lamarche et al. 1999) demonstrated directly in normolipidaemic humans that TAG enrichment of HDL particles during an Intralipid infusion enhanced the metabolic clearance of apo AI (major apolipoprotein component of HDL particle) from the circulation. The authors speculated that the increased susceptibility of TAG-rich HDL particles to lipolysis by hepatic lipase compared with fasting HDL could have accounted for the enhanced catabolism of TAG-enriched HDL apo AI. Large VLDL particles seem to be preferentially involved in the core lipid exchange mediated by CETP (Eisenberg 1985) and, therefore, the postprandial accumulation of large VLDL (Karpe et al. 1993a) could partly explain the inverse relationship between magnitude of lipaemia and fasting HDL₂ or HDL₂-cholesterol levels (Patsch et al. 1987). In line with the above reasoning, it has been suggested that the efficiency...
of postprandial TRL metabolism determines the plasma level of HDL cholesterol or larger HDL species in the postabsorptive state (Patsch et al. 1992; Miesenböck and Patsch 1992).

An alternative interpretation for the link between HDL cholesterol levels and postprandial TRL metabolism has been given by Karpe et al. (1993c) who investigated men with previous myocardial infarction. These authors observed that if the subjects were arbitrarily divided into two groups according to fasting plasma HDL cholesterol concentration below and above 1.2 mmol/L, those in the lower HDL cholesterol range showed strong, negative and linear relation between postprandial TRL responses and HDL-cholesterol concentration. In contrast, only a weak but still negative association was present in subjects in the upper HDL-cholesterol range. The authors speculated that low levels of fasting HDL-cholesterol or the larger HDL species can limit the rate of lipolytic degradation of postprandial TRL, whereas at high levels of HDL, TRL lipolytic degradation depends on factors other than HDL. This impact of a low HDL level may be mediated by decreased availability of apo CII, which impairs TRL-TAG lipolysis by LPL. This hypothesis is in a good agreement with results from a study in endurance-trained men (Cohen et al. 1991) whose HDL-cholesterol levels were well above the distribution in the general population. There was no relationship between fasting HDL-cholesterol and the postprandial TRL response. However, a subsequent study (Cohen and Grundy 1992) demonstrated that normolipidaemic men with low (~0.8 mmol/L) or normal (~1.2 mmol/L) HDL-cholesterol concentration exhibited similar postprandial lipaemia. Similar results have also been reported by O’Meara et al. (1992). These findings argue for the dissociation of postprandial TRL metabolism and fasting HDL-cholesterol, even at a low range of HDL-cholesterol concentration. It may be that high postprandial lipaemia is incompatible with high HDL-cholesterol concentrations but that low or normal postprandial lipaemia does not automatically confer high HDL-cholesterol. Accordingly, impaired TRL metabolism is not the only cause of low HDL-cholesterol levels.
2.7.3 Small dense LDL particles

LDL particles have been subfractionated with different methods after the discovery of their heterogeneous nature. For example, Austin et al. (1988) using gradient gel electrophoresis separates LDL according to the size of the particle into two classes, pattern A and B. Griffin et al. (1990) separates LDL into three subclasses using density gradient ultracentrifugation, LDL I, LDL II and LDL III.

The role of small dense LDL (defined as pattern B, LDL III or mean diameter of major peak < 25.5 nm) as a risk factor for CHD is well established (Austin et al. 1988; Griffin et al. 1994; Griffin 1995; Gardner et al. 1996). A number of potential atherogenic mechanisms have been proposed: (i) lower affinity for the LDL receptor which will effectively increase their residence time in circulation for interaction with the arterial wall; (ii) smaller size which allows faster rate of infiltration into the endothelium and subendothelial space than larger LDL; (iii) increased affinity for arterial proteoglycans; and (iv) increased susceptibility to oxidation (Griffin 1999). All these processes work together in sequence to confer increased risk on small dense LDL.

Although the LDL subclass distribution depends on genetic and environmental factors, fasting plasma TAG levels also seem to have an important effect. McNamara et al. (1992) showed a significant inverse association between change in LDL size and change in plasma TAG in a longitudinal study with a follow up period of about three years. Griffin et al. (1994) demonstrated a positive association between plasma TAG levels and the concentration of LDL III, when plasma TAG concentration was > 1.5 mmol/L. Therefore, it has been suggested that when TAG concentration exceeds this threshold level, formation of small dense LDL is facilitated (Packard 1999).

The mechanisms of small dense LDL formation in the presence of high TAG levels (Griffin 1997) resembles that of HDL particles (section 2.6.2). Briefly, it involves enrichment of LDL with TAG from TRL mediated by CETP, followed by remodeling of the TAG-enriched LDL particle by hepatic lipase. As the threshold of 1.5 mmol/L for plasma TAG is breached by postprandial lipaemia, the postprandial state could be of great importance for the formation of small dense LDL. As in the case of
HDL, large VLDL particles that accumulate postprandially act as an excellent donor of TAG to LDL and can enhance the formation of small dense LDL particles. It has also been suggested (Packard 1999) that an increase in large VLDL levels gives rise to a LDL product that has a prolonged residence time in the circulation (~5 days). This LDL particle can be then converted to small dense LDL by the action of CETP and hepatic lipase (Packard 1999). Karpe et al. (1993b) have shown that the magnitude of postprandial lipaemia is positively associated with the levels of small dense LDL in the fasted state.

2.8 Effect of high-carbohydrate diets on TAG metabolism

There has been considerable emphasis recently on reducing the fat content of the Western diet as one means to reduce CHD risk. However, low-fat diets that are necessary relatively high in carbohydrate have been implicated in the development of hypertriacylglycerolaemia. This section will discuss the effects of high-carbohydrate diets on fasting and postprandial TAG level, factors that may influence TAG response to this dietary change and potential mechanisms that may contribute to carbohydrate-induced hypertriacylglycerolaemia.

2.8.1 Effect of high-carbohydrate diets on fasting TAG concentration

A large number of studies have investigated the carbohydrate-induced increase in fasting plasma TAG levels. As this thesis is primarily concerned with effects of high-carbohydrate diets on postprandial lipaemia, the effects on fasted state will not be extensively reviewed here. Rather, some representative studies will be discussed. The interested reader is directed to the recent review paper by Parks and Hellerstein (2000).

Ginsberg et al. (1976) investigated the effects of a “control” formula diet (40 % carbohydrate, 45 % fat) and an isoenergetic high-carbohydrate formula diet (55 % carbohydrate, 30 % fat), for a week each, in 24 subjects with different TAG levels. The diets were designed to maintain body weight (euenergetic diets). Dietary carbohydrate intake was comprised of dextrins, maltose, lactose and sucrose. The same absolute amounts of sucrose were present in both diets. Both diets had a polyunsaturated to saturated fat ratio of 1 to 1 and contained 300 mg of cholesterol per day.
Consumption of the low-fat diet resulted in a mean fasting plasma TAG concentration of 41% greater than that after one week on the control diet ($P < 0.001$). When the group was divided into thirds on the basis of their plasma TAG concentration on the control diet, the per cent increment in all three groups was comparable, suggesting that the relative rise in plasma TAG concentration induced by the high-carbohydrate diet was not a function of basal TAG levels.

A meta-analysis of 27 controlled trials published between 1970 and 1991 (Mensink and Katan 1992) investigated the effect of exchanging dietary fatty acids for either other fatty acids or carbohydrates. The predicted change was that when 1% of energy as fat (regardless of saturation) is replaced by carbohydrate, fasting plasma TAG concentration would rise by about 0.025 mmol/L. For an "average" group of adult men on women with a daily energy intake of 10 MJ (2,400 kcal), 1% of energy is equivalent to about 6 g of carbohydrate or 2.7 g of fat. In an earlier study (Mensink and Katan 1987), the same authors compared the effects on plasma TAG concentration of a high-carbohydrate diet, rich in fiber (62% carbohydrate, 22% fat, 60 g fiber/day) or a high-monounsaturated fat diet (46% carbohydrate, 41% fat of which 24% monounsaturated, 43 g fiber/day) with a typical Western diet (48% carbohydrate, 38% fat of which 12% monounsaturated, 42 g fiber/day). Each diet consisted of conventional mixed solid foods and was euenergetic. Subjects consumed the Western-type diet for 17 days. Then, two groups were formed (matched for fasting TAG concentration) and for the next 36 days one group followed the high-carbohydrate, high-fiber diet and the other group followed the olive oil-rich diet. Compared to the Western diet, fasting TAG concentration rose by 0.19 mmol/L on the carbohydrate-rich diet and fell by 0.06 mmol/L on the olive oil diet ($P < 0.01$).

Ullmann et al. (1991) used a unique design to investigate whether carbohydrate-induced hypertriacylglycerolaemia can be avoided altogether if the carbohydrate content of the diet is increased gradually rather than suddenly in a single large step (such as when subjects are switched directly from diets containing 45% of energy as carbohydrate to those containing 65% of energy as carbohydrate). Eight healthy slightly overweight individuals took part and were sequentially fed five euenergetic diets: a typical American diet (40% fat, 45% carbohydrate and 15% protein) and
four carbohydrate-rich diets that were each 5% higher in carbohydrate and 5% lower in fat than the preceding diet. Phase I high-carbohydrate diet contained 50% of daily energy as carbohydrate and 35% as fat. Corresponding values for the phase IV diet were 65% and 20%. Each dietary period lasted for 10 days. Compared with the values during consumption of the typical American diet, plasma and VLDL-TAG did not change significantly during the high-carbohydrate diet at any phase. In contrast, when six of the subjects switched suddenly from the typical American diet to phase IV high-carbohydrate diet, both plasma and VLDL-TAG concentrations increased significantly. The authors concluded that the gradual phased-carbohydrate approach presumably allowed metabolic adaptation to occur without the usual stimulus of high-carbohydrate diets to enhance VLDL and TAG synthesis. Although this study has an attractive design and has been cited frequently to show that carbohydrate-induced hypertriacylglycerolaemia may be avoidable, its interesting results were not confirmed by other studies (Kasim-Karakas et al. 1997). Kasim-Karakas et al. (1997) adopted a similar design in which dietary fat was decreased stepwise from a habitual intake (33%) to 31% (phase I), 24% (phase II) and then to 14% (phase III). Corresponding values for carbohydrate intake were 51%, 53%, 60% and 67%. Each dietary period lasted four to six weeks (much longer than in the study by Ullmann et al. 1991). Plasma fasting TAG concentration rose from 1.85 mmol/L (phase I) to 2.10 mmol/L (phase II) to 2.47 mmol/L (phase III) ($P < 0.05$). These results are interesting in that the increase in TAG concentration occurred even though subjects were given longer to acclimate to the dietary change than in the study by Ullmann et al. (1991). These results have been confirmed by the same authors in a more recent study (Kasim-Karakas et al. 2000). Therefore, even with gradual increases in dietary carbohydrate intake on euenergetic diets, some individuals will experience elevations in fasting TAG concentration.

2.8.2 Effect of high-carbohydrate diets on postprandial TAG concentration

Compared to postabsorptive studies, a small number of studies have examined the effect of high-carbohydrate diets on postprandial TAG response. Furthermore, the majority of them had a “diurnal” TAG concentration approach rather than an approach of examining TAG response to a standard test meal.
In an early study, van Gent et al. (1979) compared mean diurnal TAG levels in two subjects on a high-carbohydrate-rich diet and on a high-fat diet, for two weeks each. They found that in both subjects mean diurnal TAG levels were higher after the high-carbohydrate diet. In fact, TAG levels were higher on the high-carbohydrate diet at all time points (9.00, 13.00 and 17.00 hours), regardless of the distribution of meals over day. Similar results had previously been demonstrated by Ginsberg et al. (1976). These authors found that TAG levels were significantly elevated at 8.00 hours (fasted state) and noon (prior to lunch) in 27 subjects on a high-carbohydrate formula diet (55 % carbohydrate), and although this difference became insignificant after the noon meal, postprandial TAG levels were never lower than those observed on a high-fat diet (40 % carbohydrate). In a later study (Coulston et al. 1983) of a similar design but using a somewhat more carbohydrate-rich diet (60 % carbohydrate), it was shown that plasma TAG concentrations were significantly higher on the carbohydrate-rich diet not only in the fasted state and before the noon meal but also postprandially (one and three hours after the noon meal).

A recent study (Jeppesen et al. 1997) investigated the effects of a high-carbohydrate diet (60 % carbohydrate, 25 % fat) and a high-fat diet (40 % carbohydrate, 45 % fat) on fasting and day-long TAG levels in ten healthy postmenopausal women. The two diets were euenergetic, assigned in random order and lasted for three weeks each. The ratio of sugars to starch was identical in the two diets (0.33:0.66) as was the ratio of polyunsaturated to saturated to monounsaturated fat (1.0:1.0:1.2). The amount of dietary fibre per MJ was 2.2 g for the high-fat diet and 3.25 g for the high-carbohydrate diet. Fasting plasma and VLDL-TAG concentrations were significantly higher after the subjects had consumed the high-carbohydrate diet. Also, day-long TAG concentrations in plasma and in the Sf> 400 and Sf 20-400 lipoprotein fractions were significantly higher on the high-carbohydrate diet. Vitamin A was given with the noon meal in an attempt to quantify intestinally derived TRL particles. Retinyl palmitate concentrations in plasma and the two lipoprotein fractions were significantly higher after the high-carbohydrate diet with the most substantial increase in the Sf 20–400 lipoprotein fraction, indicating postprandial accumulation of chylomicron remnants after this dietary regimen.
Using experimental diets of two-week duration and of identical composition with these in the study by Jeppesen et al. (1997), the same group reported similar results in male non-insulin dependent diabetes mellitus patients (Chen et al. 1993). The authors concluded that the postprandial accumulation of chylomicron remnants (as assessed with the retinyl palmitate method) after the 60% carbohydrate diet was possibly related to the ability of the high-carbohydrate diet to stimulate VLDL-TAG synthesis and secretion and increase the endogenous VLDL pool. This, in turn, would increase the postprandial competition of intestinal and endogenous TAG for the LPL removal pathway, impairing intestinal TAG hydrolysis and increasing the accumulation of chylomicron remnants. The same authors in a subsequent study (Chen et al. 1995) corroborated this speculation in nine non-insulin dependent diabetes mellitus patients, who followed two diets, for six weeks each, in random order: a 40% carbohydrate diet and a 55% carbohydrate diet. In both fasted and postprandial (over 24 hours) states, plasma TAG concentrations were significantly elevated after the 55% carbohydrate diet. In this study, VLDL-TAG turnover was also measured after an eight-hour fast by giving an intravenous bolus of [3H]glycerol to label endogenous VLDL-TAG. This showed that VLDL-TAG pool size was significantly increased after the high-carbohydrate diet, and this resulted from an increase in VLDL-TAG production rate as well as a decrease in VLDL-TAG fractional catabolic rate. In light of these changes, the authors attributed the augmentation of postprandial lipaemia and accumulation of chylomicron remnants observed after the high-carbohydrate to the increase in endogenous VLDL-TAG pool size and postprandial competition of TRL particles for the TAG removal pathway.

It should be noted that in all the above-mentioned studies, the test meals that were used to compare postprandial TAG responses after different diet regimes reflected the composition of the intervention diets. To the author’s knowledge, there is only one study that has compared postprandial TAG responses after a low- and a high-carbohydrate diet using an identical test meal (Blades and Garg 1995). In this study, ten male non-insulin dependent diabetes mellitus patients were fed an isoenergetic high-carbohydrate diet (55% carbohydrate, 30% fat) and a high-monounsaturated-fat diet (40% carbohydrate, 45% fat) for six weeks in a randomised, crossover design. At the end of each dietary intervention, an oral fat tolerance test was performed.
(50 g of fat labelled with retinyl palmitate, no carbohydrate) and (on a separate day) postheparin plasma LPL activity was measured after an overnight fast. The high-carbohydrate diet significantly increased fasting plasma TAG concentration but had no effect on postheparin plasma LPL activity. Incremental AUCs for plasma TAG concentrations were similar with both diets. However, visual inspection of the total AUCs (they were not reported) reveals that the high-carbohydrate diet resulted in higher total AUC, implying that this effect was due to the higher fasting TAG levels. There was no difference in the plasma retinyl palmitate response between the two diets, which, according to the authors, did not support a defect in chylomicron remnant removal. These results may suggest that carbohydrate-induced increase in post-prandial TAG levels in male non-insulin dependent diabetes mellitus patients was not related to an abnormal accumulation of chylomicron remnants. Rather, it may have been mediated by an increase in VLDL-TAG secretion in the fasted state.

2.8.3 Factors affecting TAG response to a high-carbohydrate diet
The response of fasting TAG concentrations to high-carbohydrate diets depends on several factors the most important being the energy content, duration and carbohydrate composition of the diet. These factors presumably also affect postprandial TAG concentrations as fasting and postprandial concentrations are strongly related. Factors that affect lipaemia, independently of basal TAG concentration, are difficult to be elucidated because of the limited information from postprandial studies. One factor may involve a possible interaction between the chronic effects of the background (high-carbohydrate) diet and the acute effects of the test meal. As mentioned in section 2.6.1, it has been reported that carbohydrate (fructose and sucrose) added to a fat load augments postprandial lipaemia compared to a fat-only meal (Cohen and Schall 1988; Jeppesen et al. 1995). This effect has been attributed to an increase in VLDL-TAG secretion by the liver (Cohen and Schall 1988; Jeppesen et al. 1995) or/and a decrease in TAG clearance (Grant et al. 1994). It may be that this acute effect of the carbohydrate in the test meal is amplified after a high-carbohydrate diet. Therefore, the composition of test meal could be a factor determining the postprandial TAG excursion after a high-carbohydrate diet. This is discussed in chapters 4 and 5.
2.8.3.1 Energy content of the high-carbohydrate diet

As discussed in section 2.8.1, controlled euenergetic high-carbohydrate diets, where fat is substituted with carbohydrate in order to maintain body weight, are usually associated with increases in plasma TAG concentration. However, studies in free-living individuals in which high-carbohydrate diets were consumed ad libitum rather than euenergetically, have demonstrated no change (Kasim et al. 1993; Turley et al. 1998) or significant decrease (Yu-Poth et al. 1999; Flynn et al. 1999) in fasting TAG concentration compared to typical Western diets.

It is well recognised that ad libitum high-carbohydrate diets often cause weight loss (Sheppard et al. 1991; Lichtenstein and Van Horn 1998). The lower energy density and higher volume of low-fat, high-carbohydrate diets result in automatic reductions in food and energy intakes. It seems that when consumption of high-carbohydrate diets is accompanied with weight loss, hypertriacylglycerolaemia is often avoided. A recent meta-analysis of 37 National Cholesterol Education Program Step I and Step II dietary intervention studies in free-living subjects (n > 9000), published between 1981 and 1997, showed by multiple regression analyses that for every 1 kg decrease in body weight, plasma TAG concentration decreased by 0.011 mmol/L (Yu-Poth et al. 1999). Kasim et al. (1993) reported that in 34 free-living premenopausal women taking part in a dietary counseling programme, a low-fat, high-carbohydrate diet resulted in an average weight loss of 3.4 kg by the third month, without any further decrease until the next nine months of the study. Fasting plasma TAG concentration was unchanged during the 12-month study period. A six-week high-carbohydrate diet has also been reported to result in a significant decrease in daily energy intake and body weight by 1.9 MJ and 1.5 kg, respectively, with no change in plasma fasting TAG concentration in young men (Turley et al. 1998). A recent study (Flynn et al. 1999) showed that an euenergetic National Cholesterol Education Program Step II diet significantly increased plasma fasting TAG concentration in a group of 10 men and 10 postmenopausal women but, when the same diet was administered with a 15 % energy restriction, plasma TAG concentration decreased.

A recent comprehensive study (Kasim-Karakas et al. 2000) has specifically compared the effects of a low-fat, high-carbohydrate diet under controlled euenergetic
and ad libitum conditions in a large group (n = 54) of postmenopausal women. The
dietary intervention occurred in two phases. For the first four months, participants
followed a controlled euenergetic diet in which fat intake was reduced in a stepwise
manner from 31 % to 23 % to 14 % of energy (corresponding values for carbohy­
drake intake were 53 %, 60 % and 67 %). For the next eight months, they followed an
ad libitum, self-selected, low-fat diet (12 % fat) under free-living conditions. During
the euenergetic diet, fasting plasma TAG concentration gradually increased and by
the fourth month, during the 14 % fat diet, it was (2.30 mmol/L) significantly higher
than the value at study entry (1.70 mmol/L). However, two months after subjects
switched to the ad libitum 12 % fat diet, fasting TAG concentration decreased to 1.90
mmol/L and, at the end of the 12-month study, TAG concentration (1.77 mmol/L)
was not significantly different from the baseline value (1.70 mmol/L). During the ad
libitum low-fat diet, subjects decreased their energy intake by 0.64 MJ per day,
which was accompanied by an average loss of 4.3 kg over the eight-month study pe­
riod.

2.8.3.2 Duration of the high-carbohydrate diet
There is universal agreement that euenergetic high-carbohydrate diets induce hyper­
triacylglycerolaemia in the short-term. However, controversy exists on whether this
effect is transitory or persistent.

Mancini et al. (1973) reported that the carbohydrate-induced increase in fasting
plasma TAG concentration peaked within 4-11 days and then diminished after 4-10
weeks, although it did remain above baseline values. Kashyap et al. (1982) also
showed that an euenergetic high-carbohydrate diet was associated with a rise and a
subsequent decline towards baseline in TAG concentration by the third week. An
early study in South African prisoners (Antonis and Bersohn 1961) has been widely
cited to show that changing from a low-carbohydrate (45 % carbohydrate, 40 % fat)
to a high-carbohydrate diet (70 % carbohydrate, 15 % fat) caused a rise in fasting se­
rum TAG concentration, regardless of the type of fat consumed on the low-
carbohydrate diet. TAG concentration reached a maximum (about double the starting
value) three to five weeks after the dietary change. After 20-32 weeks on the high-
carbohydrate diet most of the subjects had TAG levels similar to initial values. This
A study supported the concept that carbohydrate-induced elevation in TAG concentration is a transient phenomenon.

A study in young healthy individuals (Brussaard et al. 1982) demonstrated that fasting serum TAG concentration rose rapidly after the change-over from a control polyunsaturated fat-rich diet (31 % fat, 11 % as polyunsaturated fat) to an isoenergetic low-fat, high-carbohydrate diet (21 % fat, 4 % polyunsaturated fat) and remained stable afterwards. There was no sign of a decrease during the 13 weeks on the high-carbohydrate diet. Brussaard et al. (1982) re-examined unpublished details of the study by Antonis and Bersohn (1961). This analysis showed that the white prisoners who had initially received a 45 % fat diet rich in saturated fatty acids indeed reached baseline fasting TAG concentration by the end of the high-carbohydrate diet. In contrast, those who had started out on a 45 % fat diet rich in polyunsaturated fatty acids had serum fasting TAG concentrations that were still above the baseline value after 25 weeks on the high-carbohydrate diet, when most of them were taken off the study. According to Brussaard et al. (1982), this suggests that fasting TAG levels on a high-carbohydrate diet will probably not return to baseline level if the baseline control diet has been moderate in fat but rich in polyunsaturated fatty acids, which is agreement with the results of their own work (Brussaard et al. 1982). Jones et al. (1987) also reported significantly higher fasting TAG concentration after four months on a high-carbohydrate diet (64 % carbohydrate) than that during the baseline diet (45 % carbohydrate).

Some epidemiologic evidence suggests that the rise in plasma TAG levels associated with high carbohydrate is not transient. Studies have shown that boys from populations with high carbohydrate intakes have higher fasting TAG concentration than boys from populations with lower carbohydrate intakes (West et al. 1990; Rubinstein et al. 1988). However, it has also been reported (Connor et al. 1978) that Tarahumara Indians of Mexico have mean plasma TAG levels (~1.4 mmol/L) similar to those observed in Western populations, although they are accustomed to a 75 % carbohydrate diet. Interpretation of epidemiologic evidence with regard to an association between habitual carbohydrate intake and fasting TAG concentration is difficult as TAG levels can also be influenced by genetic factors, levels of physical activity and body fat.
2.8.3.3 Carbohydrate composition of the high-carbohydrate diet

The type of carbohydrate consumed seems to play a role in determining the TAG response to high-carbohydrate diets. Digestible forms of carbohydrate include simple sugars and starches. Indigestible forms of carbohydrate go under the name of fibre. The term "complex carbohydrate" is commonly employed as a category different from simple sugars but this term is confusing, as both starch and fibre are complex carbohydrates.

Both sucrose and fructose, given at abnormally high amounts in the diet, have been shown to raise plasma TAG concentration (Frayn and Kingman 1995). Macdonald and Braithwaite (1964) were probably the first to report that large amounts (500 g) of sucrose caused an increase in TAG levels while starch did not. Reiser et al. (1978) compared the isoenergetic exchange of sucrose with cooked wheat starch at 30 % of daily energy intake (total carbohydrate, 43 %) for six weeks each in healthy individuals. Plasma TAG concentration was (significantly) higher by 33 % with sucrose than with starch. However, from other studies, it seems that isoenergetic exchange of sucrose with starch at energy levels < 30 % has often no effect on fasting TAG level (Truswell1994). At the moment, there is no hard evidence that simple sugars are more hypertriacylglycerolaemic in humans than starches, when they are not consumed at abnormally large quantities. It can be that some subjects are more sensitive to dietary sugars than others and this predisposition could be related to the individual’s insulin sensitivity (Frayn and Kingman 1995). It is recommended that the ratio of starch to sugars is maintained at 50:50 or higher (Parks and Hellerstein 2000).

Fibre seems to protect against carbohydrate-induced hypertriacylglycerolaemia, when the diet is high in simple sugars (e.g. sucrose) (Albrink and Ullrich 1986). Abrink and Ullrich (1986) reported that the increase in fasting plasma TAG concentration, observed after 70 % carbohydrate diets providing 36 % of energy as sucrose, was partially prevented by simultaneous consumption of high levels (68 g) of fibre. Similarly, short-term consumption of a 72 % carbohydrate, high starch (62 %) diet caused increases in fasting plasma TAG concentrations that were significant only with a low (22 g), rather than high (59 g) consumption of dietary fibre (Ullrich and Albrink 1982). However, the authors concluded that the rise in TAG concentration
during the high starch diet was too small and too transient in their healthy young subjects to demonstrate a convincing protective effect of fibre against carbohydrate-induced hypertriacylglycerolaemia. In middle-aged diabetic men, Anderson et al. (1980) found a more pronounced protective effect of a high-fibre diet. In conclusion, it may be that a population more susceptible to hypertriacylglycerolaemia is more appropriate for demonstrating a preventive effect of diets high in starch and fibre.

2.8.4 Mechanisms for carbohydrate-induced increases in TAG concentration
In this section, possible mechanisms for the increases in plasma TAG concentration in response to high-carbohydrate diets are discussed. More emphasis is given in the fasted state as augmentation of postprandial TAG response due to high-carbohydrate diets is partly due to elevation of basal TAG concentration. Also, understanding of mechanisms in the fasted state (steady state) can increase understanding of mechanisms involved in the dynamic postprandial state. Potential mechanisms, independent of baseline TAG concentration, are discussed in chapters 4 and 5.

In the fasted state, most plasma TAG is carried in the VLDL fraction and two factors determine plasma levels of VLDL-TAG, namely, rates of hepatic secretion of VLDL-TAG and the capacity for hydrolysis of circulating TAG by LPL. Thus, one cause of hypertriacylglycerolaemia could be hepatic overproduction of VLDL-TAG. Another reason could be defective hydrolysis of TRL-TAG.

2.8.4.1 Hepatic overproduction of VLDL-TAG
It has been suggested that high-carbohydrate diets may increase de novo lipogenesis in the liver that, in turn, may increase fatty acid availability for esterification and hepatic TAG production (Boogaerts et al. 1984). It should be noted that there is a distinction between net de novo lipogenesis in the whole body (as evidenced by a respiratory exchange ratio > 1.0) and increased hepatic fatty acid synthesis, which may (in a net sense) be counterbalanced by fat oxidation in other tissues (for review on hepatic de novo lipogenesis, Hellerstein 1996). Using a stable isotope technique, Hellerstein et al. (1991) estimated that, in humans, de novo synthesised fatty acids contribute only approximately 1 % to VLDL-TAG fatty acids during fasting and 2 % after a high-carbohydrate mixed-meal breakfast (3.5 g carbohydrate per kg body
weight). These are fairly lifelike fasted and feeding conditions. Results from Schwarz et al. (1995) do not support a quantitatively important rate of hepatic de novo lipogenesis even after massively increased carbohydrate intake (~360 g carbohydrate added per day) for five days. Although contribution of de novo synthesised fatty acids to circulating VLDL-TAG increased more than 10-fold (1.6 % after euenergetic diet vs 20.1 % after increased carbohydrate intake), in absolute terms, less than 3.5 g fatty acids were de novo synthesised per day, representing about 12 % of the mass of VLDL-TAG secreted daily (28 g). These findings were subsequently confirmed by Aarsland et al. (1996) who investigated the contribution of de novo synthesised fatty acids to total VLDL-TAG secretion rate in healthy men in the basal state and after one and four days of a hyperenergetic high-carbohydrate diet (~2.5 times energy expenditure). Although the secretion rate of de novo synthesised fatty acids increased almost 50-fold from the basal state to day four, the 3.4-fold increase in total VLDL-TAG secretion rate was primarily due to stimulation of the secretion of preformed fatty acids. Looking at more physiological situations, Parks et al. (1999) recently demonstrated that fasting hepatic de novo lipogenesis remained low (< 5 % of VLDL-TAG fatty acids) after five weeks on an euenergetic high-carbohydrate diet (68 % carbohydrate), which was rich in complex carbohydrate and comprised whole foods. However, as pointed out by these authors, liquid diets, high in simple sugars, seem to be more lipogenic (Hudgins et al. 1996) than whole-food high-carbohydrate diets. Also, consumption of longer-chain dietary carbohydrate compared with short-chain glucose polymers and simple sugars reduce the lipogenic response to low-fat feeding (Hudgins et al. 1998).

Although hepatic de novo lipogenesis is not quantitatively the direct cause of increased VLDL-TAG production associated with high-carbohydrate diets, it may serve regulatory functions in the liver. Malonyl-CoA, the first and committed metabolite in the de novo lipogenesis pathway, plays an important role in fuel selection (McGarry and Foster 1980; Winder 1996). Specifically, it acts as a potent inhibitor of carnitine acyl-transferase I, the enzyme catalysing the transfer of long-chain fatty acids into the mitochondrial matrix for β-oxidation. In conditions of availability of surplus mitochondrial energy (such as during carbohydrate feeding) hepatic glucose metabolism is accelerated, namely, glycolytic rate, availability of pyruvate, glucose
oxidation and availability of cytosolic acetyl-CoA, which is the substrate for malonyl-CoA (Hellerstein 1996). In addition, insulin (which accompanies carbohydrate feeding) stimulates acetyl-CoA carboxylase (Mabrouk et al. 1990), the enzyme that catalyses the synthesis of malonyl-CoA from acetyl-CoA (this is the starting reaction in the de novo lipogenesis pathway). As a result, hepatic malonyl-CoA concentration increases which, in turn, inhibits β-oxidation in the liver diverting fatty acids into esterification, i.e. TAG synthesis (McGarry and Foster 1980). Therefore, the hepatic de novo lipogenesis pathway is very important in metabolic regulation because it is the source of malonyl-CoA, which determines the balance between fatty acid oxidation and esterification in the liver. If the above scenario hold true, increases in VLDL-TAG secretion attributable to a high-carbohydrate diet should be accompanied by decreases in hepatic fatty acid oxidation. Indeed, a recent study (Sidossis and Mittenddorfer 1999) demonstrated that a two-week high-carbohydrate diet resulted in significant increases in fasting VLDL-TAG production rate by ~50% with concomitant ~3-fold decreases in hepatic fatty acid oxidation. Similar findings have also been demonstrated by the same authors as a result of acute hyperglycaemia (Sidossis et al. 1998).

It has been suggested (Sniderman and Cianflone 1993) that hypertriacylglycerolaemia due to increased delivery of carbohydrate to the liver is characterised by the secretion of large, TAG-enriched VLDL without increases in the total number of particles. Mancini et al. (1973) showed that the ratio of TAG to protein in VLDL increased by 230% after a high-carbohydrate diet suggesting the formation of VLDL molecules of increased TAG content. Using dynamic light-scattering, Kasim-Karakas et al. (1997) also found a significant increase in VLDL particle size from 42.7 nm during habitual diet to 47.0 nm after a six-week 67% carbohydrate diet in healthy postmenopausal women. Melish et al. (1980) studied the kinetics of VLDL-TAG and VLDL-apo B in men (three healthy and three hypertriacylglycerolaemic) after eight days on an euenergetic fat-free liquid formula consisting of 80% carbohydrate and 20% protein. In all six subjects, the effect of carbohydrate feeding was to produce a significant increase in VLDL-TAG production rate with a group mean of 60%, which was not accompanied by an increase in VLDL-apo B production rate, suggesting the production of large, buoyant VLDL particles. However, not all studies
have found TAG-enrichment of VLDL as a response to high-carbohydrate diets (Kashyap et al. 1982), whereas other studies (Ginsberg et al. 1981; Huff and Nestel 1982) have reported significant increases of ~50% in the VLDL-apo B production rate. It seems, therefore, that high-carbohydrate diets are associated with elevations both in the amounts of TAG per VLDL and in the number of VLDL particles in plasma.

2.8.4.2 Defective hydrolysis of TRL-TAG
Using an intravenous fat tolerance test, Mancini et al. (1973) examined the TAG clearance capacity from plasma in healthy subjects fed a non-fat euenergetic formula. They concluded that peripheral hydrolysis of TAG was decreased by the high-carbohydrate formula. Two recent kinetic studies (Aarsland et al. 1996; Parks et al. 1999) in healthy subjects have reported decreases in the VLDL-TAG clearance rate after high-carbohydrate diets. In the latter study, carbohydrate-induced elevation in fasting plasma TAG concentration was attributed entirely to reduced clearance of VLDL-TAG, as production of VLDL-TAG was not found to be increased. The mechanisms responsible for reduced VLDL-TAG clearance on high-carbohydrate diets are not well defined. Carbohydrate feeding may impair VLDL-TAG catabolism by inducing alterations in the apolipoprotein composition or size of the lipoprotein. However, most of the research has focused on the effect of high-carbohydrate diets on the activity of LPL, the enzyme primarily responsible for TAG hydrolysis in circulation.

Campos et al. (1995) compared the effect of a high-carbohydrate diet (60% carbohydrate) and a low-carbohydrate diet (38% carbohydrate) for six weeks each on heparin-releasable LPL activity in 43 healthy men. Plasma TAG was significantly higher by 50% and heparin-releasable LPL activity significantly lower by 16% after the high-carbohydrate diet. LPL was inversely correlated with fasting TAG on both high- and low-carbohydrate diets (r = -0.41 and r = -0.30, respectively, P < 0.05 for both). Thompson et al. (1984) also measured heparin-releasable LPL activity and fasting plasma TAG concentration in two groups of endurance athletes before and after two weeks on either a high-carbohydrate diet (69% carbohydrate) or a low-carbohydrate diet (19% carbohydrate). Plasma TAG concentration was significantly
higher and heparin-releasable LPL activity significantly lower after the high-carbohydrate diet. When the two diet protocols were combined, there was a strong inverse relationship between changes in LPL activity and changes in plasma TAG levels ($r = -0.75$, $P < 0.01$), suggesting that changes in LPL may have mediated some of the diet-induced changes. However, two studies in non-insulin dependent diabetes mellitus patients (Chen et al. 1995; Blades and Garg 1995) do not support a lowering effect of high-carbohydrate diets on heparin-releasable LPL activity. After six weeks on a 55% carbohydrate diet, fasting plasma TAG concentration was significantly higher and heparin-releasable LPL activity was either similar (Blades and Garg 1995) or significantly higher (Chen et al. 1995) than after six weeks on a 40% carbohydrate diet.

On a tissue level, high-carbohydrate diets are believed to decrease muscle LPL activity and increase adipose LPL activity. The decreasing effect of high-carbohydrate diets on muscle LPL activity has been suggested mainly by results of two complementary studies by the same research group (Jacobs et al. 1982; Lithell et al. 1982). Subjects were normal-weight men and their diet was changed from an ordinary mixed to a high-fat diet (70% fat, 25% carbohydrate) for three days, followed by a high-carbohydrate diet (~70% carbohydrate) for another three days. Subjects body weight was maintained during the studies. The high-fat diet was preceded by one to two-hours of exercise in order to deplete the muscle vastus lateralis of its glycogen stores. Fasting biopsy samples were taken from this muscle when subjects were consuming their ordinary diet and also, after the high-fat and after the high-carbohydrate diets. Fasting serum insulin and TAG concentrations were significantly higher whereas muscle LPL activity was significantly lower after the high-carbohydrate diet than after the high-fat diet. The authors concluded that the short-term high-carbohydrate diet down-regulated muscle LPL activity possibly through higher insulin concentration. However, caution should be given before adopting these conclusions. First, the design of these studies seems to have some limitations, namely, there was no wash-out period between the experimental diets and the diet order was not randomised. Second and more important, these results were achieved under extremely different conditions of muscle glycogen status after the high-carbohydrate and high-fat diets, as a result of the exercise and dietary manipulation. Specifically,
muscle glycogen levels were supercompensated after the high-carbohydrate diet (82% higher than that on subjects' ordinary diet) whereas they were reduced by more than half after the high-fat diet. It is not clear, therefore, whether the observed differences in muscle LPL activity after the two experimental diets were due to effects of the diets per se or muscle glycogen status (as an important form of energy store) or interaction of these two. Despite the limitations of these studies, someone might hypothesise that a high-carbohydrate diet can induce a downregulation of muscle LPL, secondary to high diurnal insulin concentrations, as insulin has been shown to decrease LPL activity in skeletal muscle (Farese et al. 1991).

The effects of long-term dietary interventions on fasting skeletal muscle LPL activity have been investigated by Kiens et al. (1987) in physically trained men. They reported that a four-week high-fat diet (54% fat, 29% carbohydrate) significantly increased muscle LPL activity compared with subjects’ ordinary diet (43% fat, 42% carbohydrate). When the subjects changed to a high-carbohydrate diet (51% carbohydrate) fasting muscle LPL activity returned to “ordinary” levels and was significantly lower than after the high-fat diet. Neither of the diets affected insulin concentrations, dissociating changes in muscle LPL activity and changes in insulin levels. Serum TAG concentration was also unaffected by the experimental diets but muscle TAG concentration was significantly higher after the high-fat diet than after both the ordinary and high-carbohydrate diets. A recent report does not support an effect of low- and high-carbohydrate diets on fasting muscle LPL activity (Yost et al. 1998). These authors showed that a 16-day high-carbohydrate diet (55% carbohydrate, 25% fat) or high-fat diet (30% carbohydrate, 50% fat) did not significantly affect adipose tissue and skeletal muscle LPL activity in healthy normal-weight subjects (15 men, 10 women). In conclusion, more research is needed to determine whether carbohydrate-induced hypertriacylglycerolaemia results from impaired TRL-TAG clearance due to lower LPL activity or through some other mechanism.

2.9 Effect of exercise on TAG metabolism

Strong epidemiologic evidence indicates that there is an inverse relationship between physical activity and CHD risk (Whaley and Blair 1995). This relationship could, at least partly, be explained by the beneficial effects of exercise on lipoprotein metabo-
The best example for this is well-trained endurance athletes who exhibit blood lipid and lipoprotein profiles that are consistent with a low risk for CHD (Durstine and Haskell 1994). Determining cause and effect as a result of exercise is often complicated by changes in energy intake, diet composition, body fat and muscle mass often associated with exercise programmes. However, it appears that at least some of the effects of exercise on lipoprotein metabolism result from the exercise per se as they are seen even when exercise is not accompanied by the above mentioned changes. Exercise has a potent influence on TAG metabolism. Both repeated transitory effects of single, isolated exercise sessions and exercise-training effects are likely to be involved. This section will discuss the effects of exercise on fasting (only briefly) and postprandial TAG concentration and potential mechanisms by which exercise may exert these effects.

2.9.1 Effect of exercise on fasting TAG concentration

Endurance athletes and regular exercisers (Wirth et al. 1985) tend to have lower fasting TAG concentration than inactive individuals (Durstine and Haskell 1994, for review). In addition, levels of plasma TAG can be reduced after an aerobic training programme (Holloszy et al. 1964; Tran et al. 1983; Durstine and Haskell 1994). Weintraub et al. (1989) demonstrated a significant reduction in fasting plasma TAG concentration from 0.92 mmol/L to 0.79 mmol/L after a seven-week exercise conditioning programme in healthy young men. This reduction was accompanied by an average 43% increase in subjects' maximum oxygen uptake ($\dot{V}O_2$ max). Subjects performed, in average, four exercise sessions per week and each session consisted of 30 minutes jogging at the upper end of moderate intensity (80% of maximum heart rate). Importantly, the reduction in fasting TAG occurred, even though placing the subjects on a metabolic diet and increasing their daily energy intake during the exercise training prevented changes in diet composition and weight loss. Indeed, a meta-analysis of 95 exercise training studies showed that significant reductions in fasting TAG levels after exercise training occur even in the absence of weight loss (Tran and Weltman 1985).

A single exercise session can also attenuate fasting TAG concentration (Annuzzi et
al. 1987) (for review, Pronk 1993), although further research is needed on the short-term changes in TAG concentration in women. Generally, it seems that fasting plasma TAG concentrations are not usually altered immediately following or in the days after exercise sessions at 30-55% \( VO_2 \) max. In contrast, if exercise is prolonged and strenuous, TAG concentration is likely to be lower the day following the exercise session (Lithell 1986).

2.9.2 Effect of exercise on postprandial TAG concentration

2.9.2.1 Effect of exercise training

Several cross-sectional studies have showed that postprandial TAG response (expressed as AUC) to a test meal (40-140 g) is consistently lower in endurance-trained men (Cohen et al. 1989; Merrill et al. 1989) and women (Tsetsonis et al. 1997) than in sedentary individuals. At least in men, this was also observed when the relationship between training and postprandial lipaemia was investigated more critically by matching subjects for fasting TAG level and body weight (Cohen et al. 1989; Merrill et al. 1989). An important contribution has been made by Tsetsonis et al. (1997) who investigated differences in postprandial lipaemia between endurance trained and untrained middle-aged women in the presence and absence of effects of a recent session of moderate intensity exercise (performed ~18 hours before ingestion of the test meal). When subjects had performed the exercise session, postprandial lipaemia decreased (significantly) by 16% and 30% in the untrained and trained group, respectively. The greater decrease in the trained group may imply that the chronic and acute effects of exercise interact synergistically. Another interesting observation was that, after subjects had refrained from exercise for almost three days before the ingestion of the test meal, there was no difference in postprandial lipaemia between trained and untrained subjects. This may indicate that the residual effects of the last exercise session contribute to low postprandial lipaemia observed in endurance-trained individuals.

Cross-sectional studies using intravenous infusion of lipid emulsion to measure TAG metabolic capacity have shown that TAG removal rate is significantly enhanced in male (Sady et al. 1988; Cohen et al. 1989) and female (Podl et al. 1994) endurance athletes than in sedentary controls. Cohen et al. (1989) matched athletes with con-
tros for fasting TAG levels, however, the half-life of chylomicron-TAG was still significantly lower in athletes.

Comprehensive longitudinal studies have investigated the effects of training on post-prandial lipaemia (Weintraub et al. 1989; Aldred et al. 1995) and rate of fat clearance (Wirth et al. 1985; Thompson et al. 1988). In the study by Weintraub et al. (1989) discussed in the previous section, the 16% reduction in fasting TAG concentration was accompanied by a 37% reduction in the retinyl palmitate AUC in the chylomicron fraction, implying less postprandial competition between VLDL and chylomicrons and improved clearance of chylomicrons. The authors ascribed these reductions to changes in fitness, however, as the post-training fat tolerance test was performed 36 hours after the last training session, the possibility of an acute influence of this exercise session can not be excluded. A longer training study in young healthy men by Thompson et al. (1988), with 14 and 48 weeks of training (four to five one-hour cycling sessions per week at 80% of maximum heart rate), investigated the effect of training on TAG clearance rate. Measurement of fasting TAG concentration and intravenous fat tolerance test were performed before and after 14 or 48 weeks of training. In addition, subjects followed a controlled high-carbohydrate diet for the last four weeks before the above measurements to avoid changes in body weight and composition. After 14 weeks of exercise training, VO$_2$ max improved by 26%, fasting TAG concentration decreased by 16% and the rate of TAG clearance increased by 24%, although the latter was not significant. Continuation of training for further 34 weeks did not change VO$_2$ max or fasting TAG concentration but rate of TAG clearance increased by 49% above pre-training values, which was significant.

The studies by Wirth et al. (1985) and Aldred et al. (1995) have both attempted to exclude effects of acute exercise session by examining subjects at least 48 hours after the last training session. In the study by Wirth et al. (1985), ten male patients with primary hypertriacylglycerolaemia were trained for four months, three times per week with one-hour sessions consisting mainly jogging, ball games and callisthenics eliciting heart rate between 120 and 150 beats per minute. Body weight remained
unchanged throughout the study period whereas maximal physical performance increased after training. Although fasting TAG concentration decreased after training, the removal rate of exogenous TAG rose by only 8%. Seven of nine patients showed increased clearance after training and, therefore, the low power to detect a change may also have been a factor. In the study by Aldred et al. (1995), healthy middle-aged women trained over 12 weeks by brisk walking. Despite clear improvements in endurance fitness and body composition, there was no change in plasma TAG response to an oral fat load, compared with a control group. The authors suggested that improved endurance fitness does not necessarily result in changes in the lipaemic response above those attributable to the residual effects of the last bout of exercise. However, it was also speculated that the intensity of training (60% \(\text{\text{VAR}}_2\) max) might not have been sufficient to enhance TAG metabolic capacity. Yanes et al. (1989) found no significant difference in postprandial lipaemic response between cardiac patients who had underwent exercise rehabilitation for at least three months and those who had elected not to participate in a regular exercise programme. They attributed this to the large variability in lipaemic response in cardiac patients.

Detraining studies can be revealing about the nature of exercise training-induced changes to postprandial TAG metabolism. Mankowitz et al. (1992) studied eight healthy normolipidaemic male runners in the trained state (> 20 hours after exercise) and then after 14-22 days of detraining. These authors demonstrated that detraining reduced subjects' average \(\text{\text{VAR}}_2\) max but had no effect on their body composition, body mass and fasting TAG concentration. The impressive finding of this study was the deleterious effect of detraining on chylomicron metabolism. The retinyl palmitate AUCs in the chylomicron and chylomicron remnant fractions increased after two to three weeks of detraining by 41% \((P < 0.05)\) and 37% \((P = 0.058)\), respectively. The authors concluded that the favorable effects of exercise training on lipoprotein metabolism could decline rapidly after detraining. The effects of the early phase of detraining on postprandial lipaemia has recently been examined by Hardman et al. (1998). Ten normolipidaemic endurance-trained subjects undertook an oral fat tolerance test 15 hours, 60 hours and 6.5 days after their last training session, which was more than 30 minutes in duration. When compared with values at 15 hours, postprandial lipaemia (expressed
as total or incremental AUC) was significantly higher at 60 hours and at 6.5 days with most of the increase evident by 60 hours. Postprandial lipaemia was related to fasting TAG concentration, which followed a similar pattern over this short detraining period, i.e. it increased by an average of 47% during 6.5 days without exercise and most of this increase occurred between 15 and 60 hours after the last exercise session. These findings demonstrated the important contribution of a recent session of exercise to the low levels of postprandial lipaemia that are characteristic of endurance-trained people.

2.9.2.2 Effect of a single exercise session
Although it has been known since the early 1960s that a session of exercise diminishes postprandial lipaemia (Cohen and Goldberg 1960), it was only recently that the optimal timing to exercise (before or after a fat meal) to magnify the effect of exercise was investigated (Zhang et al. 1998). In this study by Zhang et al. (1998), twenty-one recreationally active male subjects consumed a high-fat meal on four occasions which were in a randomised order: (i), control (no-exercise, fat meal only); (ii) exercise at 12 hours before the meal; (iii) exercise at one hour before the meal and (iv) exercise at one hour after the meal. In the exercise trials, subjects performed a single exercise session at 60% of their \( \overline{VO}_2 \) max for one hour. Compared with the control trial, the AUC for TAG was 51%, 38% and 5% lower, when the exercise was performed 12 hours before, one hour before and one hour after the meal, respectively. The 5% reduction with the post-meal exercise session was not significant. These findings indicated that exercising 12 hours before the meal could magnify the lowering effect of exercise on postprandial lipaemia, implying a delayed effect of exercise on TAG metabolism. As this exercise timing seems to be the most beneficial with regard to reduction in postprandial lipaemia, this review will emphasise on studies which have used similar exercise timing.

In confirmation of the TAG-lowering effect of an exercise session performed several hours before a fat challenge (Zhang et al. 1998), Aldred et al. (1994) had previously demonstrated that postprandial lipaemia decreased by 31% when the subjects had exercised for 120 minutes at 31% \( \overline{VO}_2 \) max 15 hours before the fat intake. Gill et al.
(1998) also reported a significant reduction in postprandial lipaemia when subjects had performed 90 minutes of treadmill running at 60 % \( \bar{V}O_2 \text{ max} \) the evening prior to the test meal. Interestingly, in the study by Gill et al. (1998) the reduction in lipaemia was similar when exercise had been performed in continuous (one 90-minute session) or discontinuous (three 30-minute sessions) formats. In contrast to the results of these studies (Zhang et al. 1998; Aldred et al. 1994; Gill et al. 1998), Cohen et al. (1989) investigated the influence of an exercise session (intensity not stated) lasting 60 minutes and performed 12 hours before a fat meal in 10 sedentary men. He reported that postprandial lipaemia was slightly but not significantly reduced by exercise. This may have been because the duration and/or intensity of exercise were insufficient to induce an effect on postprandial TAG metabolism.

Tsetsonis and Hardman (1996b) investigated the effect of a 90-minute exercise session (brisk walking) of either moderate (61 % \( \bar{V}O_2 \text{ max} \)) or low (31 % \( \bar{V}O_2 \text{ max} \)) intensity, performed 15 hours prior to a fat challenge. It was shown that the postprandial TAG response (expressed as total AUC) was significantly lower (by 26 %) after the moderate exercise than in the non-exercise trial but not after the low intensity exercise. The authors suggested that the total energy expended during exercise could be an important determinant of exercise-induced decreases in postprandial lipaemia, as twice as much energy was expended during the moderate intensity exercise as during the low intensity exercise in their study (3.47 MJ vs 1.73 MJ). Indeed, in a subsequent study (Tsetsonis and Hardman 1996a), the same authors demonstrated that exercise sessions of the same energy expenditure but different intensity elicited similar attenuation in postprandial lipaemia. Specifically, they compared the effects of brisk walking, for either 90 minutes at 63 % \( \bar{V}O_2 \text{ max} \) or 180 minutes at 32 % \( \bar{V}O_2 \text{ max} \), with a non-exercise trial. The gross energy expenditure was 4.18 MJ for the low intensity and 4.28 MJ for the moderate intensity exercise session (not significantly different). However, substrate utilisation was different between the two intensities, so that oxidised fat represented 40 % and 25 % of the total energy produced during the low and moderate intensity exercise sessions, respectively \((P < 0.05)\). On the days after walking, postprandial lipaemia was reduced by both the low and the moderate
intensity walking, the magnitude of these reductions being strikingly similar (~32 %). These authors were probably the first to suggest that acute exercise-induced decreases in postprandial lipaemia could be independent of qualitative differences in substrate utilisation during exercise.

An important contribution to this issue has been made by Malkova et al. (1999). In their study, subjects underwent three oral fat tolerance tests in a balanced design. On two occasions, subjects ran on the treadmill for 90 minutes at 60 % \( \dot{V}O_2 \) max the afternoon prior the fat tolerance test, after having ingested 131 mg acipimox (antilipolytic agent) or placebo. On the third occasion they refrain from exercise (control trial). As acipimox suppresses the supply of non-esterified fatty acids from adipose tissue, NEFA levels remained suppressed during the exercise session in the acipimox trial. Although gross energy expenditure during exercise was identical in the acipimox (4.83 MJ) and placebo (4.86 MJ) conditions, the relative contributions of fat and carbohydrate was different. The proportion of energy derived from fat during exercise was lower after acipimox (16 %) than after placebo (28 %) whereas the proportion from carbohydrate was correspondingly greater (placebo 72 %, acipimox 84 %) \( (P < 0.05 \) for both comparisons). Interestingly, even after acipimox administration, fat oxidation contributed 16 % of energy expenditure during exercise, implying that intramuscular TAG was the predominant source of fatty acids for oxidation in these circumstances. Additionally, the compensatory increase in carbohydrate oxidation may have reflected a greater rate of muscle glycogen degradation. Despite the significant differences in the relative contribution of fat and carbohydrate to energy metabolism during exercise and possibly the nutrient status in muscle, postprandial lipaemia (total AUC) was strikingly similar in the two exercise trials; in both cases, ~20 % lower than in the control condition.

Since the reducing effect of exercise on postprandial lipaemia is closely related to the energy expended during exercise (Tsetsonis and Hardman 1996b, a), it is possible that the attenuation in postprandial lipaemia by exercise is attributable to the associated energy deficit rather than to the exercise per se. A recent study (Gill and Hard-
man 2000) compared the effects on postprandial lipaemia of a moderate intensity exercise session (60 % of \( VO_2 \) max) and an equivalent energy deficit induced by restriction of food intake. Compared with the control trial (no exercise, consumption of a diet isoenergetic to subjects' habitual diet), 90 minutes of brisk walking reduced postprandial lipaemia by 20 % (\( P < 0.05 \)), whereas food intake restriction (no exercise, subjects’ food intake was restricted to induce the same energy deficit, relative to control, as brought about by the 90-minute walk) reduced it by 7 % (not significantly). The authors concluded that the effect of exercise on postprandial lipaemia is greater than that attributable to the energy deficit incurred.

Studies that have investigated the effect of a single exercise session on the rate of clearance of exogenous TAG showed that clearance rate is increased the day after exercise (Sady et al. 1986; Annuzzi et al. 1987). Sady et al. (1986) demonstrated that clearance rate of an intravenous fat emulsion was increased by 76 % (\( P < 0.01 \), range 16 % to 221 %) at 18 hours after a marathon race, compared to values taken 24 hours before the race. Annuzzi et al. (1987) subsequently showed that the day after 180 minutes, but not 90 minutes, of exercise at 77 % of maximum heart rate, clearance rate of an intravenous fat emulsion was significantly increased. Gill (1999) also found no effect of a 90-minute session of moderate intensity exercise on the clearance rate of intravenous fat emulsion.

2.9.3 Mechanisms for exercise-induced decreases in TAG concentration

2.9.3.1 Enhanced hydrolysis of TRL-TAG

Studies that have investigated the hypothesis that exercise reduces plasma TAG concentration by enhancing the TRL-TAG removal pathway have mostly focused on the key enzyme, LPL. Although exercise could also affect other parts of this pathway, the existing information is on the effects of exercise on LPL activity.

Taking into consideration possible residual effects of the last exercise session, cross-sectional studies have demonstrated that endurance-trained athletes exhibit a higher post-heparin plasma LPL activity than sedentary controls (Kantor et al. 1987; Podl et al. 1994). On a tissue level, Nikkilä et al. (1978) found that LPL activity of both adipose tissue and skeletal muscle was 2.7- and 1.7-times higher, respectively, in male
long distance runners than in controls. The authors concluded that endurance training
is associated with an adaptive increase in LPL activity not only in skeletal muscle but
also in adipose tissue, which aims to increase the body capacity to mobilise and util-
ise fat as a fuel. Such an increase can allow rapid restoration of TAG stores in both
tissues, while increase in adipose tissue LPL activity can also favour release of fatty
acids directly from plasma TAG to plasma NEFA pool for oxidation in muscles
(Nikkilä et al. 1978). Higher muscle LPL activity was also present in female long
distance runners, compared with controls, but there was no difference in adipose tis-
sue LPL activity.

After seven, 15 and 48 weeks of exercise training, plasma post-heparin LPL activity
has been shown to increase by 16 %, 33 % and 49 %, respectively (Weintraub et al.
1989; Peltonen et al. 1981; Thompson et al. 1988). A number of training studies
have also demonstrated a training-induced increase in skeletal muscle LPL activity
(Lithell et al. 1981; Lithell et al. 1984; Ong et al. 1995; Seip et al. 1995) but no ef-
fect on adipose tissue LPL activity (Ong et al. 1995; Seip et al. 1995). The majority
of these studies involved daily, prolonged intense exercise, for example sessions of
more than 60 minutes duration at an intensity ranging from 60 to 75 % of \( \dot{V}O_2 \) max
(Seip et al. 1995). Detraining studies in humans have shown decreases in plasma
heparin-releasable LPL activity (Mankowitz et al. 1992), decreases in muscle and
increases in adipose tissue LPL activities (Simsolo et al. 1993). Kiens and Lithell
(1989) compared muscle LPL activity after an eight-week programme of knee exten-
sion exercise in one leg with that in the untrained leg. After training, muscle LPL ac-
tivity was 70 % higher in the trained muscle compared with the untrained muscle of
the same individual and this increase was strongly related to an increase in capillary
density. As the physiological site of LPL is the luminal surface of the capillary en-
dotheleium, the increased number of capillaries around each fibre type in the trained
compared with the untrained muscle provides more binding sites for the enzyme.
Furthermore, closer proximity of myocytes to capillaries will improve diffusion con-
ditions; and increased capacity for \( \beta \)-oxidation due to training may mean more en-
trapment of the fatty acid products of hydrolysis (Hardman 1998). All these training-
induced adaptations could explain the increased uptake of VLDL-TAG in the trained muscle that was also observed in the study by Kiens and Lithell (1989).

Acute-exercise studies have shown striking increases in plasma post-heparin LPL activity 18 hours after prolonged, intense exercise (Kantor et al. 1984; Sady et al. 1986). Elevated levels of plasma post-heparin LPL activity have also been observed in untrained and trained individuals 24 hours after one and two hours, respectively, of moderate intensity cycle ergometer exercise (Kantor et al. 1987). Herd (1997) reported a 54% (non-significant) increase in plasma post-heparin LPL activity, measured 18 hours after a two-hour moderate exercise (50% of VO₂ max). On a tissue level, one hour of exhaustive exercise has been shown to increase adipose tissue (by 44%) but not muscle LPL activity (Lithell et al. 1979a), whereas heavy exercise of longer duration (eight-hour (85 km) cross-country skiing race) did increase muscle LPL activity by 238% (Lithell et al. 1979b). In a subsequent study (Taskinen et al. 1980), it was shown that after a 20-km run (duration between 85 and 105 minutes) muscle and adipose tissue LPL activity increased by 112% and 20%, respectively, suggesting that increases in the former may be more important.

Studies that specifically investigated the exercise-induced increase in muscle LPL activity have shown that this increase usually occurs over a period of several hours (Lithell et al. 1979b) with a lag time of at least one to two hours (Lithell et al. 1979a; Kiens et al. 1989; Kiens and Lithell 1989). Kiens et al. (1989) found an increase in muscle LPL activity at four hours after 60 minutes of intense exercise, which was no longer detectable eight hours after exercise. However, studies that used exercise of longer duration (Lithell et al. 1981) reported that this increase may persist for at least 12 hours. Exercise appears to raise muscle LPL activity, at least partly, by pretranslational mechanisms, i.e. by increasing LPL mRNA level (Seip et al. 1995; Seip et al. 1997). Catecholamines, fatty acids and muscle contraction may be potential regulators of exercise-induced LPL gene expression. Epinephrine has been recently shown to stimulate human muscle LPL activity in vivo (Pedersen et al. 1999). Catecholamines increase intracellular cAMP, and the LPL promoter has a cAMP responsive element (Deeb and Peng 1989). It has also been speculated that both a fall in in-
tramyofibral concentrations of fatty acids (due to increased β-oxidation) and the muscle contractile activity per se may signal the induction of LPL gene expression (Seip and Semenkovich 1998).

2.9.3.2 Reduced hepatic production of VLDL-TAG

The evidence on a possible effect of exercise on hepatic VLDL-TAG production is sparse and limited to experimental animals. Using the Triton method, Simonelli and Eaton (1978) studied TAG production by the liver. Triton WR-1339 has detergent properties that bind plasma TAG, thereby preventing its clearance from the circulation. Hence, increments in circulating TAG after administration of Triton reflect their secretion by the liver. This study (Simonelli and Eaton 1978) showed that in obese hyperlipidaemic Zucker rats, as well as in their thin normolipidaemic littermates, hepatic TAG secretion was reduced by approximately 50% after three weeks of training (running). The authors speculated that this decrease could have been mediated by an associated reduction in the molar ratio of insulin:glucagon after training, consistent with a net catabolic change in the bihormonal state. A study by Mondon et al. (1984) has demonstrated similar results. Ten to 12 weeks of training resulted in 50% lower VLDL-TAG secretion rate in vivo. TAG secretion by perfused livers of trained and untrained rats were identical in response to given insulin and NEFA levels. This indicated that exercise training did not lead to any intrinsic difference in the ability of liver to esterify NEFA and secrete TAG, when insulin and NEFA were comparable. Rather, the lower TAG secretion rates in vivo observed in trained rats seemed to have been due to a reduction in substrate availability during the training period. In a more recent study (Fukuda et al. 1991), it was shown that the training-induced reduction in hepatic TAG secretion rate was accompanied by an increase in the production of ketone bodies. The authors concluded that the altered hepatic partitioning of long-chain fatty acids between esterification and oxidation was one of the causative factors for the serum TAG-lowering effect of exercise.

2.10 Summary

In this chapter an attempt was made to present an overview of the current knowledge of lipid and lipoprotein metabolism. In particular, literature concerning the postprandial TAG and TRL metabolism and the link between its impairment and CHD was
highlighted. A detailed description of the effects of low-fat, high-carbohydrate diets on TAG metabolism was also given, in the light of the current debate on the wisdom of recommending these diets for CHD prevention. The weight of available scientific evidence suggests that high-carbohydrate diets induce fasting hypertriacylglycerolaemia, which appears to result from overproduction of both VLDL-TAG and VLDL particles and/or an impairment in VLDL clearance. However, limited information exists on the effect of high-carbohydrate diets on postprandial TAG and TRL metabolism, which can give an insight into the atherogenic potential of these diets. Furthermore, there is sparse information on how carbohydrate-induced hypertriacylglycerolaemia can be prevented in order to allow appropriate public health recommendations to be made. The simultaneous adoption of a high-carbohydrate diet and a physically active lifestyle could be effective in this regard. Exercise, even of moderate intensity, beneficially affects TAG metabolism and available evidence for this was discussed in this chapter. The effect of high-carbohydrate diets on postprandial lipaemia and the potential role of exercise in favourably altering this effect are the main research questions of the present thesis.
CHAPTER 3
GENERAL METHODS

3.1 Subjects

All studies were conducted with the approval of Loughborough University’s Ethical Advisory Committee. Subjects were recruited from within Loughborough University and the Loughborough area by advertising in the local paper and placement of posters in the University, local shops and community centres. Volunteers were fully informed of the nature and purpose of the studies and provided with written information (Appendix A1) including possible risks and discomforts. They were also encouraged to ask questions and discuss the studies before signing a statement of informed consent (Appendix A2). Healthy, normolipidaemic young men (chapters 4 and 5) and postmenopausal women (chapter 6) were recruited. Subjects were only included in the studies if they:

a) were free of known cardiovascular disease or abnormalities, acute illness or active, chronic systemic disease.

b) had resting arterial blood pressure (mean of three readings) < 160/95 mm Hg.

c) were not taking any medication known to influence carbohydrate or lipid metabolism (except hormone replacement therapy in chapter 6 as long as it was not interrupted during the study).

d) were amenorrhoeic for > 2 years following the menopause (chapter 6).

e) were non-smokers.

f) were sedentary or recreationally active.

g) had no orthopaedic contra-indications to prolonged running (chapter 5) or walking (chapter 6).

h) had no known coagulation disorders.

i) had plasma total cholesterol concentration < 7.8 mmol.l⁻¹, fasting plasma TAG concentration < 2.3 mmol.l⁻¹, as measured in a fasting blood sample at screening.

j) had non-extreme dietary habits i.e. proportion of habitual fat intake between 25-45 % of daily total energy intake.

k) had stable body mass for the last month before the study.

l) were non-vegetarians.

At screening, to ensure they met the inclusion criteria for the studies, volunteers completed confidential health (Appendix A3) and physical activity (Appendix A4) ques-
tionnaires with the experimenter and had their arterial blood pressure, fasting plasma triacylglycerol and cholesterol concentrations measured. They were also asked to weigh and record all their food and drink intake for two week days and one weekend day (this is fully described in the “Diet recording” section).

3.2 Preliminary recording and testing

3.2.1 Diet recording

Volunteers’ habitual energy intake and diet composition were determined by the weighed food inventory method. Specifically, volunteers were asked to complete diet records (Appendix A5) by weighing and recording all their food and drink intake for two week days and one weekend day. They were specifically instructed to choose representative days to record their diet, i.e. avoid recording on days when they are involved in activities that would alter their normal diet (e.g. illness, holiday). Volunteers received written (Appendix A6) and verbal instructions and observed visual demonstrations on how to use the weighing scales and complete the diet records accurately.

3.2.2 Exercise tests

Several exercise tests were conducted in the studies described in chapters 5 and 6. Some involved running (chapter 5) and some walking (chapter 6) on a calibrated motorised treadmill (Quinton Q65 Series 90, Quinton Instrument Company, Seattle, USA). During these tests, oxygen uptake ($V_O_2$) and carbon dioxide production ($V_CO_2$) were determined and heart rate and perceived rates of exertion were recorded (all these procedures are fully described in sections 3.3, 3.4 and 3.5, respectively). All subjects were familiarised with treadmill running or walking prior to testing. During the familiarisation session the subjects ran or walked on the treadmill at different speeds and gradients and their heart rate was monitored.

3.2.2.1 Submaximal exercise testing

There is a linear relationship between $V_O_2$ and intensity of exercise at submaximal exercise intensities. Near maximum effort, $V_O_2$ plateaus and reaches the highest achievable level which is the $V_O_2$ max (Brooks et al. 1996). In the studies reported in
chapters 5 and 6, subjects undertook a four-stage incremental submaximal exercise test to determine the relationship, for each individual, between increasing treadmill speed (chapter 5) or gradient (one subject of chapter 5 and all subjects of chapter 6) and V\textsubscript{O2}. From this relationship, a linear regression equation was derived for each subject, which was used to calculate the speed or gradient necessary to elicit intensity corresponding to 60 % V\textsubscript{O2} max, as described below.

In chapter 5, the duration of this continuous submaximal test was sixteen minutes – four stages of four minutes each. The initial speed was 2.24 m/s (5 mph). Throughout the test, the treadmill’s gradient remained unchanged (zero inclination) whilst the speed increased by 0.45 m/s (1 mph) after each four-minute stage. The speed of the treadmill was elevated such that the exercise intensity increased gradually from approximately 50 % to 80 % V\textsubscript{O2} max. Expired air samples were collected and heart rate and rate of perceived exertion were recorded during the last minute of each stage. The first three minutes of every stage constituted a period of adjustment for a steady-state to be achieved. Each individual’s regression equation describing the linear relationship between V\textsubscript{O2} and speed was determined and, together with the measured V\textsubscript{O2} max (see section 3.2.2.2), the speed necessary to elicit intensity corresponding to 60 % V\textsubscript{O2} max was calculated. This calculated value was then used for the exercise sessions during the high-carbohydrate plus exercise intervention in chapter 5.

One subject (male) from chapter 5 who suffered from a knee injury and the subjects (postmenopausal women) in chapter 6 undertook a four-stage submaximal walking test during which the treadmill speed was kept constant and the gradient was increased in each stage. The duration of this test was 20 minutes - four stages of five minutes each. The speed was selected on an individual basis in order to give a comfortable, brisk walking pace. This was 2.10 m/s (4.7 mph) for chapter 5 and 1.21-1.57 m/s (2.7-3.5 mph) for chapter 6. The first stage of the test was on a level treadmill with gradient increasing by 4 % (chapter 5) or 1.5-2.5 % (chapter 6) in each subsequent stage. The increase in gradient was established on an individual basis, based on the subject’s heart rate response in the familiarisation session. Expired air samples
were collected during the last minute (chapter 5) or two minutes (chapter 6) of each stage and heart rate and rate of perceived exertion were recorded. A two-minute, rather than one-minute, expired air collection was taken in chapter 6 as the subjects were older females and their ventilation rates were relatively low. Each individual’s regression equation describing the linear relationship between $\dot{V}O_2$ and gradient was determined and, together with the predicted $\dot{V}O_2$ max (see section 3.2.2.2), the gradient necessary to elicit intensity corresponding to 60% predicted $\dot{V}O_2$ max was calculated. This calculated value was then used for the exercise sessions during the high-carbohydrate plus exercise intervention in chapter 6.

3.2.2.2 Maximal exercise testing

In chapter 5, the subjects’ $\dot{V}O_2$ max was directly determined using a modified version of the protocol devised by Taylor et al. (1955). This involved an incremental running test divided into three-minute stages, all at a constant running speed, which ranged from 2.69 m/s (6 mph) to 3.36 m/s (7.5 mph). The speed was determined individually, based on each subject’s ability to elicit test duration of between seven and twelve minutes. The starting gradient was 3.5% and increased by 2.5% in each subsequent stage. An expired air collection was taken from 1:45 to 2:45 minutes of each stage and the heart rate and perceived rate of exertion were recorded. The test was open-ended and concluded with a final expired air collection, when the subject signaled that he could only manage one more minute. The highest $\dot{V}O_2$ achieved during the test (usually the final collection) was accepted to be the subject’s $\dot{V}O_2$ max. Other criteria for achievement of $\dot{V}O_2$ max, besides volitional exhaustion, included at least three of: a respiratory exchange ratio greater than 1.1; a small or nil increase in $\dot{V}O_2$ from the penultimate to the final expired air sample (< 0.15 L/min (Taylor et al. 1955)); attainment of maximum heart rate, determined as the mean of the low estimate (220 − age) and the high estimate [210 − (0.5 × age)] (American College of Sports Medicine 1995); ventilatory equivalent for oxygen greater than 30.

In chapter 6, the subjects were women 51-66 years and had not undertaken any clinical exercise stress test to ensure no contraindications to maximal exercise. For this
reason, their $\dot{V}O_2$ max was not directly measured — as in chapter 5 — but was estimated by extrapolation of the $\dot{V}O_2$/heart rate relationship obtained during the submaximal exercise test (see section 3.2.2.1) up to the subject's predicted maximum heart rate. The maximum heart rate was estimated as described above.

3.3 Measurement of oxygen uptake and carbon dioxide production

$\dot{V}O_2$ and $\dot{V}CO_2$ were determined during all exercise tests and sessions (chapters 5 and 6) and at rest in the fasted and postprandial states (chapter 5 and 6).

Samples of expired air were collected into 50 L (for resting samples and exercise samples when volumes of expired air were low) or 150 L (for large volume samples) Douglas bags (Plysu protection Systems, Milton Keynes, UK). Subjects, whilst wearing a nose clip, breathed through a mouthpiece (Harvard Apparatus, Edenbridge, UK) fitted to a lightweight one-way respiratory valve (Jakeman and Davies, 1979), which in turn was connected to a 1.5 m length of 30 mm bore lightweight tubing (Falconia flexible ducting, Baxter, Woodhouse and Taylor Ltd., Macclesfield, UK) (exercise samples in chapters 5 and 6) or a 1.52 m length of 35 mm tubing (Hans Rudolph, inc, Kansas City, USA) (resting samples in chapters 5 and 6). The tubing terminated at a two-way valve which opened and closed the Douglas bag.

A known volume of expired air was removed from each sample to determine the percentage of oxygen using a paramagnetic oxygen analyser and of carbon dioxide using an infra-red carbon dioxide analyser (Servomex, Series 1400, Crowborough, UK). The analysers were calibrated before each sample analysis with certified reference gases (Cryoservice Ltd., Worcester, UK or Air Products, Crewe, UK).

The remaining volume of expired air in each Douglas bag was measured by evacuation through a dry gas meter (Harvard Apparatus, Edenbridge, UK). The temperature of the air in the Douglas bag was measured during evacuation using a thermistor (Edale, type 2984, Model C, Cambridge, UK) placed in the air outlet tubing of the dry
gas meter. The temperature was read from a thermometer to which the thermistor was connected.

Barometric pressure was measured using a Fortin barometer (F.D. and Company, Watford, UK) and the measured expired gas volumes were corrected to standard temperature and pressure for a dry gas (STPD) using the universal gas equation. Inspired gas volumes were derived using the Haldane transformation and \( \dot{V}O_2 \) and \( \dot{V}CO_2 \) were calculated. From these values, carbohydrate and fat utilisation rates were calculated using indirect calorimetry and the equations described by Frayn (1983) without measurement of urinary nitrogen excretion (for the estimation of protein oxidation). The contribution of amino acids to the substrate supply is normally relatively small (Brooks et al. 1996), maybe apart from during prolonged heavy exercise, and so the error in calculating carbohydrate and fat utilisation rates without measurement of urinary nitrogen excretion is probably small. Energy expenditure was determined by multiplying the calculated mass of substrates used by their respective energy densities (17 kJ/g and 39 kJ/g for carbohydrate and fat, respectively).

3.4 Heart rate
Heart rate was monitored during the preliminary tests and exercise sessions using short range telemetry (Polar PE 3000 Sport Tester or Polar Favor, Polar Electroky, Kempele, Finland).

3.5 Perceived rate of exertion
The Borg scale (Borg 1982) of perceived exertion, with numbers ranging from 6 to 20, was used to obtain each subject's own assessment of exercise intensity. This scale enabled the subjects to express their own perceived rate of exertion during the preliminary tests and exercise sessions.

3.6 Anthropometry
Anthropometric measurements were made in all studies of this thesis. These measurements were obtained according to the techniques described in The Oxford Textbook of Sports Medicine (Jones and Norgan 1994) and the Applied Body Composi-
tion Assessment (Heyward and Stolarczyk 1996). The same experimenter obtained all measurements in each study.

3.6.1 Body Mass Index

Body Mass Index (BMI) was calculated according to the formula, weight (kg) divided by the square of height (m).

Body weight to the nearest 0.05 kg was measured using a level platform beam balance (Avery Industrial Ltd., Leicester, UK), with subjects barefoot and wearing light clothing. The body mass was also taken prior to each exercise test and session for the calculation of \( \dot{V}O_2 \) per kg of body mass.

Height to the nearest 0.1 cm was also measured (with the subjects barefoot) using a Holtain fixed-wall stadiometer (Seca; Germany). The subjects stood with their heels together at a 60° angle to each other. The head was placed in the Frankfort Plane, with the line between the lower orbits of the eyes and the external auditory meati perpendicular to the vertical board. They were instructed to inhale deeply and a moveable headboard was lowered onto the top of the head with enough pressure to compress the hair. The experimenter applied vertical traction under the mastoid process and height was then measured.

3.6.2 Waist and hip circumferences

Waist and hip circumferences were measured as described by Jones and Norgan (1994) using a flexible, inelastic Fibron tape measure (CMS Weighing Equipment, London, UK). For the waist circumference measurement, the landmark was the mid-axillary line at the midpoint between the costal margin and iliac crest. This is often the natural waist. For the hip circumference measurement, subjects stood with feet together and at an angle of approximately 15°. The tape was lowered to the level of the trochanters, which is used as a guide for location of the maximum hip circumference. This measurement was made with the tape remaining horizontal, while the tape was raised and lowered until the maximum circumference was found. The waist-to-hip ratio was also calculated as an index of “body shape” by simply dividing waist by hip circumference.
3.6.3 Percentage body fat – skinfold thickness
Measurements of subcutaneous skinfold thickness were made to estimate body fatness using skinfold calipers (Holtain Ltd., Crymych, UK) and applying the prediction equations of Durnin and Womersley (Durnin and Womersley 1974). These equations are age- and sex-specific and predict body density from the logarithm (base 10) of the sum of the four skinfold thicknesses (biceps, triceps, subscapular and suprailiac). All measures were taken with the subject standing. They were repeated twice and the mean was taken as the value for the particular site.

a) Biceps – With the elbow flexed at an angle of 90°, the mid point between the lateral projection of the acromial process and the inferior margin of the olecranon process was marked. The arm was allowed to hang loosely with the palm facing forward and the measurement was made at the level of the mark over the belly of the biceps muscle above the centre of the cubital fossa.

b) Triceps – This measurement was at the same level as the biceps measurement on the midline of the posterior aspect of the arm.

c) Subscapular – This measurement was made along the natural cleavage line of the skin just inferior to the inferior angle of the scapula with the subject’s arms hanging loosely by the sides of the body.

d) Suprailiac – This skinfold was measured vertically in the mid-axillary line, half way between the costal margin and the superior iliac crest.

Each skinfold was lifted between the thumb and index finger of the experimenter’s left hand, 1 cm above the site of the measurement. The calipers were applied and the measurement was taken after 5-8 seconds of caliper pressure.

3.7 Diet analysis
Weighed food inventories were analysed using a computerised version (Comp-Eat 5.0, Nutrition Systems, London) of UK food composition tables (Holland et al. 1991). The same software was used for the prescription of the experimental diets. One limitation of this nutrient database is that, because of incomplete information, the saturated, monounsaturated and polyunsaturated fatty acid contents of many food items are estimates. For this reason, the dietary intakes of these fatty acids often did not add to 96 % by weight of the total fat intake (dietary fat contains, on average, 96 % by
weight as fatty acids; the other 4% is glycerol and other lipids (Mensink and Katan (1992)). Since the present research was designed to manipulate the intake of total fat, rather than that of fatty acids of specific saturation, the above limitation was not considered major problem. Instead, the estimates of fatty acid intake (g), as reported in the nutrient analysis, were adjusted to add to 96% by weight of the total fat intake. The percentage of energy derived from saturated, monounsaturated and polyunsaturated fatty acids was then calculated using the adjusted fatty acid intake values. These percentages are presented in chapters 4, 5 and 6.

3.8 Experimental design and main interventions
The main aim of the studies presented in this thesis was to investigate the effect of high-carbohydrate diets on postprandial TAG metabolism and how daily exercise can alter this. Postprandial TAG metabolism was studied using the oral fat tolerance test method, i.e. after the consumption of a standardised high-fat, mixed meal. In each study, the same group of subjects consumed a standardised high-fat, mixed meal on two or three (depending on the study) occasions after different interventions. These interventions involved short-term manipulation of the carbohydrate and fat, but not energy, content of subjects’ diets. Specifically, for the three days preceding each oral fat tolerance test, subjects followed a prescribed diet either high or low in carbohydrate, which was isoenergetic to their habitual diet. Subjects refrained from exercise during these interventions. The prescribed high-carbohydrate diet was also followed with the addition of daily exercise of moderate intensity (high-carbohydrate plus exercise intervention) (chapters 5 and 6). In order to remove any “order of testing” effect, a counterbalanced design was adopted, i.e. individuals in each study were randomly assigned to the interventions so that the sequence in which these were undertaken was different for different subjects. A short-term (three-day) dietary intervention model was employed because the lipoproteinemic changes are likely to be more marked shortly after the initiation of a high-carbohydrate diet than after longer periods of intervention during which adaptive processes may counterbalance the initial effects. Therefore, the initial, dynamic period of dietary change, when effects on lipoprotein metabolism are becoming manifest, may be revealing with regard to carbohydrate-induced hypertriacylglycerolaemia and its potential prevention by exercise. There was a 10-day wash-out interval between interventions. Full details of the
experimental design and main interventions are presented in the methodology section of each experimental chapter.

3.8.1 Experimental diets
All experimental diets employed in this thesis were designed to be isoenergetic, on an individual basis, to subjects' previously determined habitual energy intake (section 3.2.1), in order to maintain subjects' body mass. In an attempt to ensure this, subjects were specifically instructed to record their habitual diet on representative days and explained in detail how to complete the diet records accurately (section 3.2.1). It is known that under-recording often occurs when the self-reported energy intake method is used (Livingstone et al. 1990; Kasim-Karakas et al. 2000). For this reason, daily energy intake values determined from the subjects' diet records were compared with those obtained using energy requirement prediction equations which take into consideration subjects’ gender, age, body mass and physical activity level (Department of Health 1991). For a few subjects who reported unrealistically low daily energy intake, the predicted, rather than self-reported, daily energy requirement was used for prescription of the experimental diets.

All experimental diets comprised normal foods, excluding alcohol. The sources of carbohydrates were cereals, pasta, rice, fruits, breads, vegetables, honey, jam, and biscuits. The sources of fat were commercial margarines, oils, avocado pears, nuts, dairy products, and meats. In chapter 4, as the composition of the diets was particularly extreme, the food items were prescribed on an individual basis based on each subject’s preferences. Subjects in chapters 5 and 6 consumed the same food items. A spreadsheet (Microsoft Excel 97) was used to establish the amount in grams for each food item according to each subject’s previously determined daily energy intake. The experimenter kept in close contact with subjects, who were encouraged to contact her if they had any questions at all concerning the experimental diets.

Three meals and one or two snacks were consumed each day, prepared by the subjects themselves. The food items were provided by the researchers (apart from chapter 4), together with detailed instructions about the mass of each item to be consumed and methods of preparation and cooking. Subjects were instructed to follow the ex-
perimental diets "to the gram" and weigh and record each item consumed. Full description of the composition of the diets is presented in the methodology section of each experimental chapter.

3.8.2 Exercise sessions
In the high-carbohydrate plus exercise intervention, subjects performed an exercise session on the treadmill of either 30 (chapter 5) or 60 minutes (chapter 6) daily. Each exercise session was carried out in the laboratory at an intensity of about 60 % \( \dot{V}O_2 \) max, which is considered as moderate by most classifications. For instance, according to the American College of Sports Medicine exercise intensity classification system (American College of Sports Medicine 1995), exercise within the range of 50 to 74 % \( \dot{V}O_2 \) max is moderate. Regular exercise of moderate intensity confers significant health benefits and is recommended by various scientific bodies for the prevention of CHD and for cardiac rehabilitation programmes (American College of Sports Medicine 1995; NIH Consensus Conference 1996). In addition, exercise at this intensity is associated with a lower risk of injury, acute cardiac events during exercise or discontinuation of activity than vigorous exercise (NIH Consensus Conference 1996).

For the group of young men studied in chapter 5, exercise at 60 % \( \dot{V}O_2 \) max meant running. For the group of older postmenopausal women studied in chapter 6, exercise at this intensity meant brisk walking. The treadmill speed and gradient necessary to elicit intensity corresponding to 60 % \( \dot{V}O_2 \) max was calculated during the preliminary testing, as described in section 3.2.2. The intensity of exercise was monitored by collecting one- (chapter 5) or two-minute (chapter 6) expired air samples (section 3.3) every 15 minutes. Heart rates (section 3.4) and perceived rates of exertion (section 3.5) were also recorded during exercise.

3.8.3 Preparation for the main interventions
In all studies, preceding each three-day intervention, subjects' diets and physical activity were standardised as described in the individual chapters.
3.9 Test meal protocol
Subjects arrived at the laboratory after a 12-hour overnight fast, at approximately 08:00 hours. A cannula was introduced into a forearm or antecubital vein and the subject rested quietly in a supine position for 10 minutes, after which a baseline blood sample was obtained. The test meal (see section 3.9.1) was then consumed over a median of 11 (range 9 – 14) minutes. Further blood samples were obtained (with subjects in the supine position) 15, 30, 45, 60 and 90 minutes after completion of the meal, and then hourly until 6 hours. The cannula was kept patent by flushing with non-heparinized saline (9 g/L). Expired air samples were also collected (chapters 5 and 6) for 6-minute periods in the fasted state and postprandially (every hour) using Douglas bags, for measurement of oxygen uptake and carbon dioxide production (see section 3.4). Subjects rested or performed sedentary activities (working quietly, watching television, reading, etc.) during the observation period and consumed only mineral water. This was provided *ad libitum* on the first test and replicated during subsequent tests.

3.9.1 The test meal
The test meal employed in the studies of this thesis was a high-fat, mixed meal and a modification of the meal described previously by Schlierf *et al.* (1987). It was given according to body mass (Table 3.1) and its macronutrient composition is shown in Table 3.2. Table 3.3 presents the energy and macronutrient content of the test meal (chapters 4 and 5) for a subject of 77.3 kg.

The reproducibility of this test meal has recently been examined (Gill 1999). It was shown that the mean difference ± SD in the postprandial TAG response (total AUC) on a test-retest basis was 2.0 ± 13.3 %. This indicates that this test has probably enough precision to detect exercise-induced decreases in postprandial lipaemia, which are often more than 20 % when for instance a 90-minute exercise session of moderate intensity is employed 15 hours prior to the test meal (Gill 1999).
<table>
<thead>
<tr>
<th></th>
<th>Chapters 4 and 5 (per kg body mass)</th>
<th>Chapter 6 (per kg body mass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whipping cream (g)</td>
<td>2.53</td>
<td>2.11</td>
</tr>
<tr>
<td>Oats (g)</td>
<td>0.75</td>
<td>0.63</td>
</tr>
<tr>
<td>Brazil nuts (g)</td>
<td>0.15</td>
<td>0.12</td>
</tr>
<tr>
<td>Milk chocolate (g)</td>
<td>0.13</td>
<td>0.11</td>
</tr>
<tr>
<td>Sultanas (g)</td>
<td>0.13</td>
<td>0.11</td>
</tr>
<tr>
<td>Desiccated coconut (g)</td>
<td>0.07</td>
<td>0.06</td>
</tr>
<tr>
<td>Granny Smith apple (g)</td>
<td>0.67</td>
<td>0.56</td>
</tr>
<tr>
<td>Banana (g)</td>
<td>1.13</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Table 3.1. Quantities of ingredients of the test meal, administered per kg body mass.

All ingredients were purchased from J Sainsbury's.

<table>
<thead>
<tr>
<th></th>
<th>Chapters 4 and 5 (per kg body mass)</th>
<th>Chapter 6 (per kg body mass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ)</td>
<td>65.8</td>
<td>54.6</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>1.22</td>
<td>1.01</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>1.11</td>
<td>0.92</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>0.18</td>
<td>0.15</td>
</tr>
<tr>
<td>Fat (% energy)</td>
<td>69</td>
<td>69</td>
</tr>
<tr>
<td>Carbohydrate (% energy)</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Protein (% energy)</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 3.2. Macronutrient composition of the test meal.
Table 3.3. Energy and macronutrient content of test meal for an example subject, body mass 77.3 kg.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (MJ)</td>
<td>5.08</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>85</td>
</tr>
<tr>
<td>Sugars</td>
<td>54</td>
</tr>
<tr>
<td>Starch</td>
<td>31</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>93</td>
</tr>
<tr>
<td>Saturated</td>
<td>57</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>26</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>6</td>
</tr>
<tr>
<td>Cholesterol, mg</td>
<td>205</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>14</td>
</tr>
<tr>
<td>Fibre, g</td>
<td>14</td>
</tr>
</tbody>
</table>
3.9.2 Preparation for the test meal
Subjects were instructed to come to the laboratory for the test meal after a 12-h fast. They were also instructed to drink plenty of water the evening prior to the test meal to prevent dehydration. They arrived at the laboratory by car to ensure they were at the rested state.

3.10 Blood sampling
Venous blood samples were obtained via an indwelling cannula (Venflon 2, 18G/45 mm: BOC Ohmeda AB, Helsingborg, Sweden) placed in an antecubital or forearm vein or occasionally by venopuncture of an antecubital vein. For cannulation, subjects lay on an examination couch and a small volume of local anaesthetic (1 % w/v lignocaine hydrochloride; Antigen Pharmaceuticals Ltd., Roscrea, Ireland) was injected subcutaneously. The cannula was then introduced and a 10-cm three-way stopcock (Connecta, BOC Ohmeda AB, Helsingborg, Sweden) was attached for blood sampling. The cannula was kept patent by flushing with saline solution (0.9 %; B. Braun Ltd., Buckinghamshire, UK). All blood samples were obtained with subjects in the supine position (for at least 15 minutes) and were collected directly into Monovettes (Sarstedt Ltd., Leicester, UK) via a “multi-adaptor” (Sarstedt Ltd., Leicester, UK). Different types of Monovettes were used depending on the subsequent analysis. Serum Monovettes were used to collect samples for determination of insulin and 3-hydroxybutyrate. Samples to be analysed for glucose and lactate were collected into pre-chilled sodium-fluoride Monovettes. All other blood samples were collected into pre-chilled potassium ethylenediamine tetra-acetic acid (EDTA) Monovettes. Sodium-fluoride and potassium-EDTA Monovettes were kept chilled until centrifugation.

Blood samples collected into sodium-fluoride and potassium-EDTA Monovettes were centrifuged (Koolspin; Burkard, Scientific Ltd., Uxbridge, UK) within 15 min of collection for 15 minutes at 1,250 g at 4°C. Blood collected into serum Monovettes was allowed to clot for 1 h at room temperature before centrifugation for 15 minutes at 1,250 g at 4°C. Plasma aliquots were stored at -20°C and serum aliquots at -70°C for later analysis. In chapter 6, one plasma aliquot (4 mL) was kept at 4°C, until a TRL fraction was separated and apo B48 and B100 concentrations were deter-
mined. To minimise proteolytic degradation of apo B in this aliquot, 1.0 μL per mL plasma of phenylmethylsulfonylfluoride (PMSF, Sigma) 10 mM dissolved in isopropanol and 5 μL per mL plasma of aprotinin (Trasylol, Bayer, Leverkusen, Germany) 1.4 μg/L were added.

3.11 Analysis of blood samples
These analyses were conducted in the Biochemistry Laboratory in the Department of Physical Education, Sports Science and Recreation Management unless otherwise stated.

3.11.1 Spectrophotometric assays
Concentrations of TAG in plasma (all chapters) and the TRL fraction (chapter 6) were measured enzymatically using a commercially available kit (Roche Diagnostics Ltd., Lewes, UK) (chapter 4) or the method described by the Oxford Lipid Metabolism Group (Humphreys et al. 1990) with correction for free glycerol (Appendix B1) (chapters 5 and 6). In all studies, plasma was also analysed for total cholesterol, glucose, lactate (Roche Diagnostics Ltd., Lewes, UK) and non-esterified fatty acids (NEFA) (Wako, Wako Chemicals GmbH, Neuss, Germany) by standard enzymatic, colorimetric methods. A magnesium chloride/phosphotungstic acid precipitation method (Roche Diagnostics Ltd., Lewes, UK) was used to isolate an HDL-containing supernatant from plasma which was subsequently analysed for cholesterol. Serum 3-hydroxybutyrate concentrations were determined by an enzymatic method (Sigma Diagnostics, Poole, Dorset, UK). All spectrophotometric assays were performed on an automated analyser (Cobas Mira Plus, Roche Diagnostic Systems, Hertfordshire, UK). The plasma TAG concentrations in chapter 4 were determined on Cobas Bio, Version 8326.

3.11.2 Radioimmunoassays
Concentrations of serum insulin were determined using a solid-phase 125Iodine radioimmunoassay (COAT-A-COUNT; Diagnostic Products, Los Angeles, CA, USA). Radioactivity was measured using an automated gamma counting system (Cobra II, Packard Instrument, Downers Grove, IL) (Appendix B2). This analysis was conducted in the Chemistry Department, Loughborough University.
3.11.3 Apolipoprotein E phenotyping

Apolipoprotein E phenotype was determined by Dr SJ Mastana and Mrs A Pacynko in the Human Genetics Laboratory (Department of Human Sciences, Loughborough University), by isoelectric focusing using Western blot techniques (Eichner et al. 1991).

3.11.4 TRL fractionation

The TRL fraction (Sf > 20) was separated by preparative ultracentrifugation. The TRL fractionation was conducted on fresh plasma the day after the samples were collected. The plasma had been mixed with a preservative cocktail (see section 3.11.4) and stored overnight at 4°C. During the analyses the samples were chilled in ice trays.

Approximately 4.2 mL of 1.006 g/mL density solution (prepared by dissolving 11.0 g NaCl in 995 ml distilled water and adding 0.1 mL of 0.1 mmol/L EDTA) was injected at the bottom of bell-topped centrifuge tubes (13 x 51 mm) (Quick-Seal, Beckman Instruments Ltd., High Wycombe, Bucks, UK) using a 21G x 3.81 cm needle (Sabre International Products Limited, Reading, UK) attached to a 5-mL syringe (Plastipak, Becton Dickinson, Oxford, UK). The tubes were weighed. Approximately 2 mL of plasma was then carefully layered underneath the density solution using a cannula-needle (Venflon 2, 18G/45 mm: BOC Ohmeda AB, Helsingborg, Sweden) attached to a 2 mL syringe (Plastipak, Becton Dickinson, Oxford, UK) and the tubes were re-weighed and topped up with density solution. The tubes were then sealed with a Beckman tube topper kit (Beckman Instruments Inc., London, UK) and gently placed in a fixed-angle rotor (Beckman, TLA 100-4). They were centrifuged for 150 min in an Optima TLX (Beckman Instruments Ltd.) at 100,000 rpm (average 543,000 g) at 4°C. The tubes were then placed in a Beckman Centri-Tube slicer (Beckman Instruments Ltd.) and sliced at 3.5 cm from the bottom to isolate the TRL fraction. This was aspirated into pre-weighed 3 mL polycarbonate tubes (Fisher Scientific, Loughborough, UK) using a 21G x 3.81 cm needle attached to a 2 mL syringe. The tube slice and the tube slicer blade were then rinsed with density solution to dislodge TRL particles which were stuck to the walls of the tube slicer.
and this liquid was added to the TRL suspension in the polycarbonate tube. The tube was then re-weighed to determine the mass of the TRL suspension.

The concentration (mmol/L) of TAG in the TRL fraction suspension was then determined. This concentration was multiplied by the mass of the fraction (assuming the density of the fraction to be 1 g/mL) and divided by 1000 to give the mmolar quantity of TAG in each fraction. This was then multiplied by 1000 and divided by the original volume of plasma used to generate the fraction to give the concentration (mmol/L) of TRL-TAG in the plasma sample.

3.11.5 Determination of TRL apo B48, apo B100 and apo E concentrations
Apo B48, apo B100 and apo E in the TRL fraction were quantified using analytical SDS-PAGE as described by Karpe and Hamsten (1994). This is a type of electrophoresis used to separate proteins by molecular weight. The proteins are reduced and denatured and SDS is used to give proteins a consistent charge-to-mass ratio. These analyses were conducted by the author under the supervision of researchers in the Oxford Lipid Metabolism Group.

Aliquots of the TRL fraction were delipidated in a methanol-diethyl ether solvent system. The delipidations were always performed immediately after ultracentrifugation. A volume of 3.0 mL of methanol (99.8 %, BDH Analar) was vigorously added to 200-300 μL of TRL fraction in a 7 mL round-bottom glass tube with a polyethylene stopper (Vacutainer®, Vacutainer Systems, Becton Dickinson, UK). A volume of 3.0 mL diethyl ether (99.5 %, BDH Analar) was then vigorously added to the tube and the delipidation mixture was centrifuged (GS-15R, Beckman Instruments Ltd.) for 25 minutes in a swing-out rotor (S4180, Beckman Instruments Ltd.) at 4°C. After gentle removal of the solvent, another 3.0 mL diethyl ether was immediately added and the sample was centrifuged for another 25 minutes under the same conditions, whereafter the diethyl ether was discarded. The tube was allowed to dry until a whitish haze was seen at the bottom of the tube. The protein sediments were solubilised in 50 μL of sample buffer (2 % SDS, 0.15 M sodium phosphate, 12.5 % glycerol, 5 % mercaptoethanol, and 0.001 % bromphenol blue at pH 6.8) by mixing for 3×3 minutes at room temperature. The dissolved protein mixture was subsequently denatured
at 80°C for 10 min. After denaturation the tubes were immediately centrifuged (GS-15R, Beckman Instruments Ltd.) for 2 minutes in a swing-out rotor (S4180, Beckman Instruments Ltd.). Samples were then frozen at -20°C until analytical SDS-PAGE was performed.

Gradient SDS gels (3-27 %) (ICN Biomedicals, Inc, Ohio, USA) were used for electrophoresis and apo B100 from LDL (0.2 μg/μL) (provided by the Oxford Lipid Metabolism Group) was used as a reference protein and for standard curve dilutions. When gels were run simultaneously and thereafter fixed, stained, and destained in the same bowl, a standard curve was applied to only one of these gels. For the construction of the standard curve, five amounts of the apo B100 standard were applied (0.1 μg, 0.2 μg, 0.5 μg, 1 μg and 1.5 μg). Electrophoresis was performed using a vertical BioRad Miniprotein II apparatus connected to a BioRad Power Pack 300 supply. The upper and lower electrophoresis buffers contained 25 mM Tris, 192 mM glycine, and 0.2 % SDS adjusted to pH 8.5. Pre-electrophoresis was performed for 15 min at 60 V. Normally, both 2 μL and 10 μL of each sample were applied to obtain at least one protein band within the range of the apo B100 standard curve. Gels were run first at 60 V for 15 min and then at 100 V for approximately 1 hour until the bromophenol blue bands reached the lower end of the gel. After electrophoresis, gels were fixed in 12 % trichloroacetic acid for 30 min, then stained in 0.2 % Coomassie G-250 (Merck, Poole, UK), 40 % methanol, and 10 % acetic acid for 3 hours, and finally destained in 12 % methanol, 7 % acetic acid for 48 hours with four to five changes of destainer. Gels were scanned using BioRad GS700 Imaging Densitometer. The background was subtracted by scanning an empty lane.

The apo B 48 and B100 mass in the 10 μL and 2 μL application, respectively, were calculated using the apo B100 standard curve, as the chromogenicity of these apolipoproteins has been found to be equal (Karpe and Hamsten 1994). For the calculation of TRL apo B48 and B100 concentrations in the plasma sample, several dilution factors were taken into consideration: (i) the total volume of sample buffer (50 μL) that the delipidated TRL fraction was dissolved in divided by the sample volume applied to the gel; (ii) the volume of the TRL fraction recovered divided by the volume of TRL fraction delipidated (usually 0.3 mL for fasting samples and 0.2 mL for post-
prandial samples); and (iii) I divided by the volume of plasma ultra-centrifuged to
get TRL apo B48 and B100 concentrations in the plasma sample in mg/L. Apo E was
quantified using the apo B100 standard curve by taking account of the difference in
chromogenicity by multiplying with 2.6 (Björkegren et al. 1997).

3.11.6 Determination of remnant-like lipoprotein particle cholesterol concentra-
tion

The RLP-cholesterol was determined in serum samples using a novel immunosepa-
rature kit technique (Jimro-II) made available from the Japanese Immunoresearch
Laboratories Company (Nakajima et al. 1996). This analysis was conducted by the
author under the supervision of researchers in the Oxford Lipid Metabolism Group.

Serum was mixed with a lipoprotein separation medium consisting of a Sepharose gel
suspension to which monoclonal (Mab) antibodies directed against apo B100 (JIH)
and apo AI had been attached (Nakajima et al. 1993). The Mab JIH recognises an
epitope in the region of apo B51 (Nakajima et al. 1996). It binds to all apo B100-
containing particles (i.e. VLDL and LDL) but not to a minor fraction of VLDL that is
enriched in apo E and has remnant characteristics (Campos et al. 1992). This anti-
body also fails to recognise apo B48-containing particles. When the kit is used, all
HDL and (newly-secreted) chylomicrons containing apo AI are precipitated. The un-
bound fraction contains chylomicron remnants and apo E-enriched VLDL particles
that have properties resembling VLDL remnants. In normolipidaemic subjects likely
to have low levels of TRL remnants, apo E-containing HDL-sized lipoproteins may
also be found in the unbound fraction (Marcoux et al. 1998). It may be that these
lipoproteins do not contain neither apo B nor apo AI and thus are not recognised by
the immunoaffinity gel antibodies (Marcoux et al. 1998). The unbound fraction there-
fore contains a large proportion of RLP particles and, in some conditions, lipoprotein
particles of smaller size that may not be direct products of TRL lipolysis. The subse-
quent measurement of the cholesterol content of the unbound fraction is likely to
provide a good estimate of remnant cholesterol levels in serum and could be used as a
marker for RLP. The best diagnostic marker for RLP has been evaluated and among
cholesterol, triacylglycerol, phospholipids and apo B measurements, cholesterol was
found to be the most sensitive, precise and convenient (Nakajima et al. 1993). In addition, cholesterol measurement is widely used to estimate atherosclerotic risk.

Fasting RLP-cholesterol concentration has been shown to be strongly associated with serum TAG concentration (Nakajima et al. 1993) and the concentration of apo B48 and apo B100 TRL particles (Karpe et al. in press). The increase in RLP-cholesterol after fat intake has been correlated with the increase in TRL apo B48 and large apo B100 TRL particles (Karpe et al. in press). Despite the strong association between plasma TAG and RLP-cholesterol concentrations, there is considerable interindividual variability in RLP-cholesterol levels and subjects with the same TAG concentration often have very different RLP-cholesterol levels (Nakajima et al. 1993).

Age, gender and menopausal status have significant effects on RLP-cholesterol concentrations. RLP-cholesterol concentrations increase with age in both men and women (Nakajima et al. 1993; McNamara et al.1998). Data from the Framingham Heart Study (McNamara et al.1998) also showed that postmenopausal women (n = 718) had significantly higher mean RLP-cholesterol levels than premenopausal women (n = 667) (0.188 mmol/L vs. 0.163 mmol/L). However, women (n = 1385) consistently had lower mean concentrations than men (n = 1436) (0.176 mmol/L vs. 0.208 mmol/L). From the data that were generated, ranges for low, normal and increased RLP-cholesterol concentrations were calculated for fresh samples. These were: low, < 0.15 mmol/L; normal, 0.15-0.25 mmol/L; and increased, > 0.25 mmol/L.

The RLP-cholesterol kit consists of the following reagents:

- Immunoaffinity gel (30 mL): mouse monoclonal antibodies to anti-human apo B100 (J1-H) and anti-human apo Al (H-12) conjugated to Shepharose 4B beads as the solid phase.
- Wash buffer (750 mL): 0.01 M Tris-HCL buffer with 0.01 % EDTA, 0.02 % sodium azide, pH 7.4.
- Cholesterol reagent R1 (40 mL) containing ascorbate oxidase, cholesterol esterase and cholesterol oxidase.
- Cholesterol reagent R2 (10 mL), containing horseradish peroxidase, 4-aminoantipyrine and N-ethyl-N-(3-methylphenyl)-N’-succinyl-ethylenediamine.
• Cholesterol-ester calibrator 1.292 mmol/L (2 mL).

According to the manufacturer’s instructions, serum is the preferable sample for the assay but EDTA or heparin plasma may also be used. Concentrations in fasting or non-fasting EDTA plasma samples have reported to be higher than in serum or heparinised plasma (Leary et al. 1998). Freezing may result in higher RLP-cholesterol concentration. It has been calculated that at an RLP-cholesterol concentration of 0.25 mmol/L, there was a positive bias of 0.023 mmol/L, producing a concentration of 0.273 mmol/L (McNamara et al. 1998). The separation of the RLP fraction is sufficient provided the total TAG concentration in the sample is < 11.3 mmol/L; HDL-cholesterol, < 2.3 mmol/L; and LDL-cholesterol; < 5.2 mmol/L. The detection limit of the assay is estimated to be 80 µmol/L or 0.080 mmol/L.

The immunoaffinity Sepharose gel was prepared (after inversion to ensure complete suspension) by centrifuging the required amount for 1 min at 800 rpm at 4°C. The supernatant was removed and equal volume of wash buffer was added and the tube was suspended. The tube was again centrifuged for 1 minute under the same conditions, the supernatant was removed and equal volume of wash buffer was added. The tube was suspended and the immunoaffinity gel suspension was ready for use.

Two Hitachi micro-sample cups (supplied by JIMRO) were labeled for each sample. A 3.2-mm diameter steel bead (provided by JIMRO) was added to each cup using a bead dispenser (provided by JIMRO). A total of 300 µL of the immunoaffinity Sepharose gel suspension and 10 µL of serum were then added to each cup. The separation medium was gently mixed (RLP Mixer, J-100, Photol, Otsuka Electronics, Japan) for 120 minutes at room temperature. The RLP mixer has a series of magnetic bars driving the steel bead up and down in each cup to ensure mixing during the incubation (Nakajima et al. 1996). The RLP separation gel was then allowed to settle for at least 20 minutes, but less than 30 minutes.

The cholesterol content in the supernatant was assayed in duplicate enzymatically using a centrifugal autoanalyser (Monarch, Instrumentation Laboratory, Warrington, UK). Calibrators of different concentrations (103 µmol/L, 52 µmol/L, 26 µmol/L, 13
$\mu$mol/L, 6.5 $\mu$mol/L and 3.2 $\mu$mol/L) were used for construction of a calibration curve. These were prepared by serial dilutions of the cholesterol ester calibrator (1292 $\mu$mol/L) in wash buffer. For the calculation of RLP-cholesterol concentration in the serum sample, the cholesterol in the supernatant was multiplied by 31 to correct for the dilution of serum sample at the immunoseparation step.

### 3.12 Calculations and statistics

Plasma, TRL and serum concentrations measured in the fasted state were compared using Student's t-test (chapter 4) or one-way analysis of variance (ANOVA) for repeated measures (chapters 5 and 6) when two or three interventions were employed, respectively. Summary measures (Matthews et al. 1990) of the postprandial responses were calculated as the total areas under plasma, TRL fraction and serum concentration vs time curves (AUC) using the trapezoidal rule. The AUC is a useful way of summarising the postprandial response for each individual from the series of measurements before and after the meal. Summary measures were compared using student t-test or one-way ANOVA, again depending on the number of interventions employed. In addition, when the pattern of change over time in the postprandial response seemed different, the interaction effect of intervention and time was determined using two-way ANOVA for repeated measures. This analysis was proven particularly important in situations where the AUCs after different interventions were similar but there was a clear difference in the pattern of the response, indicating a qualitative, rather than quantitative, effect of the interventions on the postprandial response. Data were checked for normality before statistical analyses were performed using the Kolmogorov-Smirnov test. When data were not normally distributed, they were transformed to their natural logarithm. Normal distribution of transformed values was confirmed before statistical testing. If significant differences were identified by ANOVA, Tukey post hoc comparisons were performed to determine where these differences occurred. Relationships between variables were evaluated using Pearson's product moment correlation coefficient. A 5% level of significance was adopted throughout. Statistical procedures were performed using Statistica for Windows, version 5.0 (Tulsa, OK, USA).
3.13 Accuracy and precision

The accuracy and precision of the assays described in the sections above were monitored using quality control sera; for glucose and lactate, Precirorm U (Roche Diagnostics Ltd., Lewes, UK); for TAG, Precirorm L (Roche Diagnostics Ltd., Lewes, UK) and Control Serum N (Human) (Roche Diagnostics Ltd., Lewes, UK); for total and HDL-cholesterol, Precirorm L (Roche Diagnostics Ltd., Lewes, UK); for NEFA, Seronorm Lipid (SERO AS, Asker Norway); for 3-hydroxybutyrate, β-HBA Control Normal (Sigma Diagnostics, Poole, UK).

All samples from each subject were analysed in the same batch, except for TAG determination in the TRL fraction (chapter 6), where analyses were performed in fresh plasma. Within-batch coefficient of variations were 1.3 % for plasma TAG with correction for free glycerol (Humphreys et al. 1990) (chapters 5 and 6) and 1.2 % using the commercially available kit (Roche Diagnostics Ltd., Lewes, UK) (chapter 4), 0.7 % for cholesterol, 1.8 % for HDL cholesterol, 0.8 % for glucose, 1.3 % for lactate, 0.9 % for NEFA, 1.4 % for 3-hydroxybutyrate and 4.2 % for insulin. The within-batch coefficient of variation for TRL-TAG determination was 5.4 % and for apo B-48 and apo B-100 determinations were 4.4 % and 4.2 %, respectively. The between-batch coefficient of variation for TAG measurement using the method described by Humphreys et al. (1990) was 3.3 % at 0.250 mmol/L and 1.6 % at 0.9 mmol/L. Quality control samples (provided by the Oxford Lipid Metabolism Group) prepared by human serum were used for the RLP-cholesterol assay. The within-batch coefficient of variation was 7.7 % at 0.20 mmol/L and 6.6 % at 0.64 mmol/L.
CHAPTER 4
POSTPRANDIAL LIPAEMIA AFTER SHORT-TERM VARIATION IN
DIETARY FAT AND CARBOHYDRATE

4.1 Introduction
There is divergence of opinion on the optimal macronutrient recommendation for protection against CHD. Whereas the reduction of dietary saturated fat forms a well-accepted tenet, the controversy focuses on which macronutrient can be the best replacement. The two main contenders are complex carbohydrates and unsaturated fat. Replacement of saturated with unsaturated fat has been shown to have effects on lipoprotein metabolism which would be expected to reduce the risk of CHD (Katan 1997; Katan et al. 1997). However, distrust of the strategy to replace fat with fat (Connor and Connor 1997; Ornish 1998; Rudel 1998) has shifted the focus of recommendations to diets low in fat and high in carbohydrates. In the UK, for example, a desirable level likely to decrease the incidence of diet-related chronic disease has been set at 33% of energy from fat (Department of Health 1991), a reduction of 5% compared with figures for 1996 (Ministry of Agriculture Fisheries and Food 1997).

Replacement of fat with carbohydrates has been recommended because this reduces plasma total and LDL-cholesterol concentrations (Clevidence et al. 1992). Recently, however, concern has been expressed that low-fat, high-carbohydrate diets may not confer the anticipated benefits for cardiovascular disease risk (Baum and Brown 2000) because such diets also increase fasting plasma TAG and decrease HDL-cholesterol concentrations (Mensink and Katan 1992). According to epidemiological evidence, these alterations may be expected to increase the risk of CHD (Hokanson and Austin 1996). High-carbohydrate diets have also been associated with the predominance of small dense LDL particles (Dreon et al. 1999) that are potentially atherogenic (Griffin 1999).

Despite the fact that they contain less fat, high-carbohydrate diets increase postprandial lipaemia (Jeppesen et al. 1997; Coulston et al. 1983; Chen et al. 1993). This effect appears to be of great importance. First, an exaggerated TAG response, espe-
cially at late time points, has been recognized as an independent marker of CHD (Patsch et al. 1992). Second, the magnitude of postprandial lipoaemia shows a strong inverse association with fasting HDL-cholesterol concentration (Patsch et al. 1983) and a positive association with the preponderance of small and dense LDL particles (Karpe et al. 1993). As discussed in sections 2.7.2 and 2.7.3, an exaggerated post-prandial lipoaemia probably enhances the opportunity for exchange of HDL- and LDL-cholesterol esters for TAG from TRL particles, resulting in the formation of cholesterol-depleted HDL and small dense LDL particles. Thus, one may speculate that exaggeration of postprandial lipoaemia could, at least partly, explain the reduction in HDL-cholesterol concentration (Katan 1998) and shift in LDL particle mass from predominantly larger to predominantly smaller LDL particles (Dreon et al. 1999) which have been reported in healthy volunteers in response to a low-fat, high-carbohydrate diet.

Consequently, it is important that the links between dietary change and postprandial TAG metabolism are understood and their implications for the atherosclerotic process explored. In the majority of postprandial studies investigating the effect of high-carbohydrate diets (Jeppesen et al. 1997; Coulston et al. 1983; Chen et al. 1993), the test meals were not standard but reflected the composition of the background diets which were compared. Therefore, the test meal on each occasion had different composition as it contained different amount of carbohydrate and fat. In this way, these studies do not distinguish the acute effects on postprandial lipoaemia of the test meal from the chronic effects of the preceding diet.

It has been suggested that carbohydrate-induced hypertriacylglycerolaemia is a short-term adaptive response to an increase in dietary carbohydrate and that this effect diminishes after a couple of weeks (Bierman 1995; Truswell 1994). On the other hand, results from experimental (Brussaard et al. 1982) and epidemiological (West et al. 1990) studies show that this can be a long-lasting phenomenon. Either way, the lipoproteinemic changes are likely to be more marked shortly after the initiation of a high-carbohydrate diet than after longer periods of intervention during which adaptive processes may counterbalance the initial effects. Carbohydrate-induced hyper-
triacylglycerolaemia may thus be best examined through study of the initial responses to a switch to a high-carbohydrate diet.

The present study was, therefore, designed to investigate the effects of short-term manipulation of the fat and carbohydrate content of isoenergetic diets on the metabolic responses to a standard test meal. An additional objective was to establish a short-term dietary intervention model for future investigations of the hypertriacylglycerolaemic effect of high-carbohydrate diets and, importantly, its prevention. In order to provide a clear picture of differences in postprandial events, the experimental diets employed had distinctly different composition.

4.2 Subjects and methods

4.2.1 Subjects

Nine normolipidaemic men took part in the study, which was approved by the Loughborough University Ethical Advisory Committee. All subjects were fully informed of the procedures and risks involved and gave their written consent. Some physical characteristics and plasma concentrations of TAG, total cholesterol and HDL-cholesterol of subjects as measured after an overnight fast on recruitment, whilst subjects were following their habitual lifestyle are presented in Table 4.1. All subjects were non-smokers and none had any physician-diagnosed cardiovascular or metabolic disease or was taking drugs known to influence lipid or carbohydrate metabolism. To exclude individuals with extreme habitual dietary practices, only non-vegetarian subjects and subjects with habitual fat intakes constituting between 25% and 45% of energy intake were accepted. Subjects' habitual diet was assessed by the weighed food inventory method over two weekdays and one weekend day, as described in section 3.2.1. Information about the energy content and macronutrient composition of subjects' habitual diets is presented in Table 4.2. Seven subjects were recreationally active in moderate exercise (e.g. fast walking, easy cycling) three times per week. Two also engaged in more strenuous exercise, again around three times per week. Five subjects possessed the E3/3 phenotype, two the E3/2 and two E4/3.
<table>
<thead>
<tr>
<th>Physical characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, year</td>
<td>26.7 ± 3.8</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>79.6 ± 8.2</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.78 ± 0.04</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.1 ± 1.8</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>84.0 ± 6.7</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.86 ± 0.05</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>18.9 ± 4.3</td>
</tr>
<tr>
<td>TAG, mmol/L</td>
<td>0.93 ± 0.29</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.18 ± 0.94</td>
</tr>
<tr>
<td>HDL-cholesterol, mmol/L</td>
<td>1.08 ± 0.19</td>
</tr>
</tbody>
</table>

Table 4.1 Physical characteristics of subjects and plasma concentrations of lipoprotein lipids measured in the fasted state. $n = 9$ men, mean ± SD.
4.2.2 Study design
Each subject consumed a standardised high-fat, mixed meal, with an interval of 13 days, after two different interventions. These interventions comprised: (i) three days on a high-fat diet and (ii) three days on a high-carbohydrate diet. The order of the interventions was counterbalanced.

4.2.3 Experimental diets
The experimental diets were isoenergetic and their energy value approximated, on an individual basis, subjects' previously determined energy intake (sections 3.2.1 and 3.8.1). They were based on normal foods, excluding alcohol, and their energy and macronutrient contents are shown in Table 4.2. Three meals and one or two snacks were consumed each day. Subjects prepared these themselves, to a menu agreed with the experimenters. This included detailed instructions about the mass of each item to be consumed and methods of preparation and cooking. Subjects were instructed to follow the experimental diets "to the gram" and were also asked to weigh and record each item consumed. Examples of the prescribed high-carbohydrate and high-fat diets are presented in Table 4.3 and Table 4.4, respectively. Subjects refrained from exercise during the three-day interventions; only activities of daily living and slow walking for personal transport were permitted. Caffeine consumption was not restricted during the interventions but subjects were instructed to standardise it for the day prior the oral fat tolerance tests.

During the three days leading up to the first intervention period, diets were unrestricted but subjects weighed and recorded all foods consumed, replicating this prior to the subsequent intervention. During these three days, they also refrained from exercise and from alcohol consumption. Otherwise, subjects returned to their usual lifestyle during the 10-day wash-out interval.

4.2.4 Diet analysis
Weighed food inventories were analysed for energy and major nutrients using a computerised version (Comp-Eat 5.0, Nutrition Systems, London) of UK food composition tables (Holland et al. 1991).
4.2.5 Test meal protocol

Full details of the test meal protocol were given in section 3.9. Briefly, subjects arrived at the laboratory after a 12-h fast at approximately 0800 h. A cannula was introduced into a forearm or antecubital vein and the subject rested quietly in a supine position for 10 minutes, after which a baseline blood sample was obtained (details on blood sampling are given in section 3.10). The test meal (described in detail in section 3.9.1) was then consumed. The meal was given according to body mass (per kg of body mass: 1.2 g fat, 1.1 g carbohydrate, 0.2 g protein). For these subjects, this meant 96 ± 3 g fat, 93 ± 3 g carbohydrate, 14 ± 1 g protein and 5.54 ± 0.19 MJ of energy, 69 % of which came from fat. Further blood samples were obtained (with subjects in the supine position) 15, 30, 45, 60 and 90 minutes after completion of the meal, and then hourly until 6 hours. Subjects rested or worked quietly during the observation period and consumed only water. This was provided ad libitum on the first trial and replicated during the second trial.

4.2.6 Analytical methods

At each sampling point in the fasted and postprandial states, blood samples for plasma preparation were separated within 15 minutes of collection, divided into aliquots and were stored at -20°C. At most time points, separate samples were collected for serum preparation. These were allowed to clot at room temperature before separation and were stored at -70°C. Plasma samples were analysed for total and HDL-cholesterol (fasted and 6-h samples only), TAG, NEFA, glucose and lactate, and serum samples for 3-hydroxybutyrate (all by enzymatic, colorimetric methods). Serum samples were also analysed for insulin using a solid-phase ¹²⁵I radioimmunassay. Details of these analyses have been given in section 3.11. Phenotypes of apolipoprotein E were determined by isoelectric focusing, using Western blot techniques.
<table>
<thead>
<tr>
<th></th>
<th>Habitual diet</th>
<th>High-carbohydrate diet</th>
<th>High-fat diet</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy, MJ</strong></td>
<td>11.7 ± 1.7</td>
<td>12.2 ± 2.1</td>
<td>11.9 ± 2.1</td>
</tr>
<tr>
<td><strong>Carbohydrate, %</strong></td>
<td>50 ± 9</td>
<td>68 ± 3</td>
<td>18 ± 4</td>
</tr>
<tr>
<td><strong>Sugars</strong></td>
<td>20 ± 6</td>
<td>35 ± 5</td>
<td>6 ± 2</td>
</tr>
<tr>
<td><strong>Starch</strong></td>
<td>30 ± 9</td>
<td>33 ± 4</td>
<td>12 ± 2</td>
</tr>
<tr>
<td><strong>Fat, %</strong></td>
<td>35 ± 9</td>
<td>18 ± 3</td>
<td>66 ± 5</td>
</tr>
<tr>
<td><strong>Saturated</strong></td>
<td>13 ± 5</td>
<td>8 ± 1</td>
<td>24 ± 3</td>
</tr>
<tr>
<td><strong>Monounsaturated</strong></td>
<td>13 ± 4</td>
<td>6 ± 1</td>
<td>22 ± 2</td>
</tr>
<tr>
<td><strong>Polyunsaturated</strong></td>
<td>8 ± 4</td>
<td>3 ± 1</td>
<td>17 ± 2</td>
</tr>
<tr>
<td><strong>Cholesterol, mg</strong></td>
<td>319 ± 140</td>
<td>200 ± 89</td>
<td>352 ± 74</td>
</tr>
<tr>
<td><strong>Protein, %</strong></td>
<td>15 ± 3</td>
<td>14 ± 1</td>
<td>16 ± 3</td>
</tr>
<tr>
<td><strong>Fibre, g</strong></td>
<td>25 ± 11</td>
<td>22 ± 8</td>
<td>18 ± 5</td>
</tr>
</tbody>
</table>

Table 4.2 Energy content and composition of the habitual and experimental (high-carbohydrate and high-fat) diets. Each experimental diet was consumed for three days. \( n = 9 \) men, mean ± SD.
High-carbohydrate diet

Total energy: 11.3 MJ
CHO : FAT : PROTEIN
69% : 18% : 13%

<table>
<thead>
<tr>
<th>Breakfast</th>
<th>Amount, g</th>
<th>Actual amount, g</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bran flakes</td>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Semi-skimmed milk</td>
<td>200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White bread</td>
<td>80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jam</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange juice</td>
<td>240</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coffee with semi-skimmed milk</td>
<td>50 (milk)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lunch</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White bread</td>
<td>160</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flora, 38% vegetable fat</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean ham</td>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange juice, unsweetened</td>
<td>240</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Banana</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Snack</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swiss roll</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange juice, unsweetened</td>
<td>240</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dinner</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White spaghetti, boiled</td>
<td>260</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheddar cheese, medium</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flora 38% vegetable fat</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swiss roll</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Snack</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tango orange drink</td>
<td>660</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digestive biscuits, plain</td>
<td>45</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please eat and drink **everything** that has been prescribed for the three days of the high-carbohydrate diet. Please refrain from exercise on these three days and **do not** eat or drink anything else (except water).

Table 4.3 An example of the prescribed high-carbohydrate diet. It was consumed for three days.
## High-fat diet

Total energy: 11.3 MJ  
CHO : FAT : PROTEIN  
20% : 69% : 11%

<table>
<thead>
<tr>
<th>Breakfast</th>
<th>Amount, g</th>
<th>Actual amount, g</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bran flakes</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Semi-skimmed milk</td>
<td>200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coffee with semi-skimmed milk</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Snack</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potato crisps</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peanuts</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lunch</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wholemeal bread</td>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flora, 70% vegetable fat</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cream cheese</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet 7UP</td>
<td>Free</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cucumber, lettuce</td>
<td>Free</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peanuts</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dinner</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuna, canned in oil, drained</td>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White spaghetti, boiled</td>
<td>130</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mayonnaise</td>
<td>140</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheddar cheese, medium</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cucumber, lettuce</td>
<td>Free</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Snack</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Semi-skimmed milk</td>
<td>150</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please eat and drink everything that has been prescribed for the three days of the high-fat diet. Please refrain from exercise on these three days and do not eat or drink anything else (except water).

Table 4.4  An example of the prescribed high-fat diet. It was consumed for three days.
4.2.7 Calculations and statistics
Plasma and serum concentrations measured in the fasted state were compared using Student's t-test for correlated means. Total postprandial responses were calculated as the AUC using the trapezoidal rule. The incremental postprandial TAG response was calculated as the total AUC minus the fasting value extrapolated over six hours. These summary measures (section 3.12) were compared between interventions using Student's t-test for correlated data. In addition, the interaction effect of intervention and time was determined using two-way ANOVA for repeated measures. Relationships were evaluated using Pearson's Product Moment correlation coefficient. A 5% level of significance was adopted throughout and results are expressed as mean ± SEM, unless otherwise stated.

4.3 Results

4.3.1 Experimental diets and test meal
Both experimental diets were well tolerated, aside from a common complaint by the subjects of feeling excessively full during the high-carbohydrate diet. Most subjects also complained for the high-fat diet being excessively rich. Compliance, assessed by food inventories and detailed discussions with subjects, was high. The test meal was also well tolerated by all subjects without any sign of nausea or other gastrointestinal discomfort. It was consumed over an average of 11.4 minutes (both interventions).

4.3.2 Plasma and serum concentrations in the fasted state
Plasma concentrations of TAG, lactate and serum concentration of insulin were significantly higher, whereas HDL-cholesterol and NEFA were significantly lower after the high-carbohydrate diet than after the high-fat diet, (Table 4.5). No significant differences were found for glucose or total cholesterol. Serum 3-hydroxybutyrate concentration was somewhat lower after the high-carbohydrate diet (P = 0.06).

4.3.3 Postprandial responses
The total AUC (Figure 4.1, upper panel, Table 4.6) was higher after the high-carbohydrate diet than after the high-fat diet and the pattern of change in TAG concentration differed between interventions (interaction of intervention × time, P < 0.001). Peak concentration occurred later after the high-carbohydrate diet (3-4 h post-
prandially, compared with 1-2 h) and at 6 h was still significantly higher than the fasting value \( (P < 0.001) \). The total lipaemic response (AUC) was closely related to fasting TAG concentrations in the high-carbohydrate intervention \( (r = 0.97, P < 0.01) \) but not in the high-fat intervention \( (r = 0.61, \text{NS}) \). The incremental lipaemic response (Figure 4.1, lower panel, Table 4.6) was higher after the high-carbohydrate diet and the pattern of change over time also differed between interventions (interaction of intervention \( \times \) time, \( P < 0.001 \)).
Table 4.5

<table>
<thead>
<tr>
<th></th>
<th>High-carbohydrate</th>
<th>High-fat</th>
<th>P value¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAG, mmol/L</td>
<td>1.18 ± 0.18</td>
<td>0.62 ± 0.09</td>
<td>0.02</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>3.98 ± 0.25</td>
<td>3.81 ± 0.25</td>
<td>0.14</td>
</tr>
<tr>
<td>HDL-cholesterol, mmol/L</td>
<td>1.01 ± 0.08</td>
<td>1.10 ± 0.09</td>
<td>0.002</td>
</tr>
<tr>
<td>Non-esterified fatty acids, mmol/L</td>
<td>0.21 ± 0.03</td>
<td>0.30 ± 0.04</td>
<td>0.003</td>
</tr>
<tr>
<td>3-hydroxybutyrate, mmol/L</td>
<td>0.02 ± 0.01</td>
<td>0.07 ± 0.02</td>
<td>0.06</td>
</tr>
<tr>
<td>Insulin, μIU/mL</td>
<td>10.9 ± 0.8</td>
<td>9.2 ± 0.6</td>
<td>0.03</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.13 ± 0.13</td>
<td>5.26 ± 0.13</td>
<td>0.15</td>
</tr>
<tr>
<td>Lactate, mmol/L</td>
<td>1.08 ± 0.09</td>
<td>0.73 ± 0.03</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Concentrations measured in the fasted state for plasma TAG, total cholesterol, HDL cholesterol, non-esterified fatty acids, glucose, lactate, and serum 3-hydroxybutyrate and insulin, after the high-carbohydrate diet and after the high-fat diet. Each diet was consumed for three days. n = 9 men, Mean ± SEM.

¹ Significance of differences between trials by Student's t-test for correlated means.
Figure 4.1  Total (upper panel) and incremental (lower panel) plasma concentrations of TAG in the fasted state (0 hour) and for six hours following consumption of a high-fat, mixed meal after three days on a high-carbohydrate diet (High-CHO) and after three days on a high-fat diet (High-fat). $n = 9$ men. Mean ± SEM.
Postprandial responses of insulin, NEFA and 3-hydroxybutyrate, glucose and lactate are shown in Figure 4.2, Figure 4.3 and Figure 4.4, respectively. Summary measures of these responses are presented in Table 4.6. The serum insulin response did not differ between interventions, nor did the change in concentration over time. The plasma glucose response was lower ($P = 0.009$) after the high-carbohydrate diet and the plasma lactate response was higher ($P = 0.001$). The change in plasma concentrations of glucose and lactate over time did not differ between interventions. The pattern of change of NEFA over time was clearly different (interaction of intervention $\times$ time, $P < 0.001$); the concentration was lower after the high-carbohydrate diet in the fasted state and for four hours postprandially, but higher thereafter. The serum 3-hydroxybutyrate response to the high-fat meal was significantly lower ($P = 0.02$) after the high-carbohydrate diet, with a much smaller rise between two and six hours than after the high-fat diet (interaction of intervention $\times$ time, $P < 0.001$).

Neither total nor HDL-cholesterol changed over the observation period in either intervention. In the 6-h blood samples, plasma total cholesterol did not differ significantly between interventions but, as in the fasted state, HDL-cholesterol was lower after the high-carbohydrate diet ($1.00 \pm 0.08$ mmol/L vs $1.10 \pm 0.09$ mmol/L, $P = 0.002$).
Figure 4.2 Serum concentrations of insulin in the fasted state (0 hour) and for six hours following consumption of a high-fat, mixed meal after three days on a high-carbohydrate diet (High-CHO) and after three days on a high-fat diet (High-fat). \( n = 9 \) men. Mean ± SEM.
Figure 4.3  Concentrations of plasma non-esterified fatty acids (NEFA) (upper panel) and serum 3-hydroxybutyrate (lower panel) in the fasted state (0 hour) and for six hours following consumption of a high-fat, mixed meal after three days on a high-carbohydrate diet (High-CHO) and after three days on a high-fat diet (High-fat). \( n = 9 \) men. Mean ± SEM.
Figure 4.4  Plasma concentrations of glucose (upper panel) and lactate (lower panel) in the fasted state (0 hour) and for six hours following consumption of a high-fat, mixed meal after three days on a high-carbohydrate diet (High-CHO) and after three days on a high-fat diet (High-fat). \( n = 9 \) men. Mean ± SEM.
Table 4.6

<table>
<thead>
<tr>
<th></th>
<th>High-carbohydrate diet</th>
<th>High-fat diet</th>
<th>P value(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total TAG, mmol/L × h</td>
<td>12.63 ± 1.64</td>
<td>6.64 ± 0.50</td>
<td>0.003</td>
</tr>
<tr>
<td>Incremental TAG, mmol/L × h</td>
<td>5.54 ± 0.64</td>
<td>2.92 ± 0.47</td>
<td>0.002</td>
</tr>
<tr>
<td>Non-esterified fatty acids, mmol/L × h</td>
<td>2.40 ± 0.20</td>
<td>2.80 ± 0.21</td>
<td>0.12</td>
</tr>
<tr>
<td>3-hydroxybutyrate, mmol/L × h</td>
<td>0.23 ± 0.06</td>
<td>0.62 ± 0.11</td>
<td>0.02</td>
</tr>
<tr>
<td>Insulin, μIU/mL × h</td>
<td>144 ± 34</td>
<td>143 ± 25</td>
<td>0.99</td>
</tr>
<tr>
<td>Glucose, mmol/L × h</td>
<td>31.5 ± 1.0</td>
<td>33.6 ± 1.1</td>
<td>0.009</td>
</tr>
<tr>
<td>Lactate, mmol/L × h</td>
<td>6.40 ± 0.43</td>
<td>4.96 ± 0.22</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Six-hour areas under the plasma or serum concentration vs time curves for TAG, non-esterified fatty acids, 3-hydroxybutyrate, insulin, glucose and lactate after three days on a high-carbohydrate diet and after three days on a high-fat diet. \(n = 9\) men. Mean ± SEM.

\(^1\) Significance of differences between trials by Student's t-test for correlated means.
4.4 Discussion
This study was designed to investigate the effects of manipulation of the fat and carbohydrate content of isoenergetic diets on the metabolic responses to a standard test meal. It was conducted during the initial, dynamic period of dietary change when effects on lipoprotein metabolism are becoming manifest. The findings confirm that low-fat, high-carbohydrate diets increase fasting (Mensink and Katan 1992) and postprandial (Coulston et al. 1983; Jeppesen et al. 1997; Chen et al. 1993) plasma TAG concentrations, with a complementary decrease in HDL-cholesterol. However, in the postprandial studies just referred to, the test meal employed after each diet intervention was not standardised with regard to its macronutrient composition. Specifically, it reflected the composition of the background diet and thus contained different amounts of fat and carbohydrate on each occasion. Therefore, the differences in the postprandial responses to the test meals encompassed both the acute effects of the macronutrients in the meals and the chronic effects of the preceding diets. In the present study, the same test meal was used after each dietary intervention and therefore the differences in the responses to the meal reflected the effects of the antecedent diets per se and maybe also their interactions with the macronutrients in the standard test meal.

The major new finding is that postprandial lipaemia in healthy men was 90% higher after the short-term high-carbohydrate diet than after the high-fat diet, with differences in TAG concentration between trials of up to 0.9 mmol/L. This finding, alongside the marked differences in the associated postprandial metabolic milieu, suggests that the present three-day dietary model may be attractive for future investigations of carbohydrate-induced hypertriacylglycerolemia and of interventions which might attenuate this.

In the fasted state, diet-induced differences in VLDL secretion may have contributed to the 0.6 mmol/L difference between interventions in plasma TAG concentration. High-carbohydrate diets have been shown to stimulate hepatic synthesis and secretion of VLDL-TAG (Sidossis and Mittenddorfer 1999), generating an expansion of the fasting TAG pool (Jeppesen et al. 1997; Gonen et al. 1981). Increased hepatic de novo lipogenesis may occur during high-carbohydrate feeding but is not likely to be
4.4 Discussion
This study was designed to investigate the effects of manipulation of the fat and carbohydrate content of isoenergetic diets on the metabolic responses to a standard test meal. It was conducted during the initial, dynamic period of dietary change when effects on lipoprotein metabolism are becoming manifest. The findings confirm that low-fat, high-carbohydrate diets increase fasting (Mensink and Katan 1992) and post-prandial (Coulston et al. 1983; Jeppesen et al. 1997; Chen et al. 1993) plasma TAG concentrations, with a complementary decrease in HDL-cholesterol. However, in the postprandial studies just referred to, the test meal employed after each diet intervention was not standardised with regard to its macronutrient composition. Specifically, it reflected the composition of the background diet and thus contained different amounts of fat and carbohydrate on each occasion. Therefore, the differences in the postprandial responses to the test meals encompassed both the acute effects of the macronutrients in the meals and the chronic effects of the preceding diets. In the present study, the same test meal was used after each dietary intervention and therefore the differences in the responses to the meal reflected the effects of the antecedent diets per se and maybe also their interactions with the macronutrients in the standard test meal.

The major new finding is that postprandial lipaemia in healthy men was 90% higher after the short-term high-carbohydrate diet than after the high-fat diet. This finding, alongside the marked differences in the associated postprandial metabolic milieu, suggests that the present three-day dietary model may be attractive for future investigations of carbohydrate-induced hypertriacylglycerolemia and of interventions which might attenuate this.

In the fasted state, diet-induced differences in VLDL secretion may have contributed to the 0.6 mmol/L difference between interventions in plasma TAG concentration. High-carbohydrate diets have been shown to stimulate hepatic synthesis and secretion of VLDL-TAG (Sidossis and Mittenddorfer 1999), generating an expansion of the fasting TAG pool (Jeppesen et al. 1997; Gonen et al. 1981). Increased hepatic de novo lipogenesis may occur during high-carbohydrate feeding but is not likely to be the main determinant of increased VLDL-TAG secretion. As discussed in detail in
section 2.8.4.1, results from several studies do not support a quantitatively important rate of hepatic *de novo* lipogenesis, even after massively increased carbohydrate intake (Schwarz *et al.* 1995). However, *de novo* lipogenesis due to surplus carbohydrate energy plays an important role in fuel selection (McGarry and Foster 1980; Winder 1996) through malonyl-CoA which inhibits β-oxidation in the liver, channeling fatty acids into esterification, i.e. TAG synthesis (McGarry and Foster 1980). It has, therefore, been proposed that carbohydrate-induced hypertriacylglycerolaemia occurs because of increased intracellular hepatic availability of fatty acids for TAG synthesis, stemming from inhibition of their oxidation by high glucose uptake (Sidossis and Wolfe 1996). This hypothesis is in line with the four-fold decrease in hepatic fatty acid oxidation that has been reported after a high-carbohydrate diet, alongside a 50 % increase in VLDL-TAG production rate (Sidossis and Mittenddorfer 1999).

In addition to the potential effects of the high-carbohydrate diet on VLDL, suppressive effects of the high-fat diet on VLDL secretion may have contributed to the difference between interventions in fasting TAG concentration. Studies in rat hepatocytes have found that a high-fat diet decreases VLDL production, partly because increased oxidation of fatty acids decreases their availability for VLDL-TAG synthesis (Francone *et al.* 1992; Oussasou *et al.* 1996). Both mechanisms, i.e. suppression of hepatic fatty acid oxidation after a high-carbohydrate diet and/or enhancement after a high-fat diet, would be consistent with the observed difference between interventions in fasting serum 3-hydroxybutyrate (Table 4.3). 3-Hydroxybutyrate (a ketone body) is produced by the liver and, since ketone bodies production, under normal conditions, occurs during increased hepatic fatty acid β-oxidation, serum 3-hydroxybutyrate concentration can be used as a marker of hepatic fatty acid β-oxidation. However, the higher serum 3-hydroxybutyrate concentration after the high-fat diet could reflect, in addition, reduced availability of oxaloacetate, which is required for the acetyl-CoA to enter into the tricarboxylic acid cycle (Stryer 1995). Oxaloacetate can be used as a gluconeogenic precursor during carbohydrate restriction, and subjects in the present study could be considered as carbohydrate-restricted after the high-fat intervention. Their daily carbohydrate intake during the high-fat intervention was on average 135 g, which is less than the minimum amount of carbohydrate required daily (200 g) (Macdonald 1999) and also about 2.5-fold less than their habitual carbohydrate intake (369 ± 93 g).
In the fasted state the hydrolysis of VLDL-TAG may also have been influenced by the experimental diets, amplifying differences in TAG concentration between interventions. In line with this hypothesis, it has recently been reported (Parks et al. 1999) that VLDL-TAG clearance in humans in the fasted state was reduced after a low-fat, high-carbohydrate diet. As discussed in section 2.8.4.2, there are also reports which demonstrated that increases in fasting plasma TAG concentration due to high-carbohydrate diets were accompanied by significant decreases in plasma heparin-releasable LPL activity (Thompson et al. 1984; Campos et al. 1995), implying a defect in plasma TAG hydrolytic capacity after this dietary regime.

In the postprandial state, an exaggerated and protracted lipaemic response was observed after the high-carbohydrate diet. This will have been partly due to the expanded fasting TAG pool as chylomicrons compete with VLDL for the same, rate-limiting enzyme (LPL) for hydrolysis of their core TAG (Brunzell et al. 1973; Karpe and Hultin 1995; Björkegren et al. 1996). Therefore, an increase in the fasting VLDL concentration due to the high-carbohydrate diet would increase the competition between TRL particles of exogenous and endogenous origin postprandially, leading to impaired hydrolysis of their TAG content and an exaggeration in TAG response to the test meal. This however may not be the sole explanation for the difference in postprandial lipaemia.

The composition of the test meal employed may have also been a factor determining the difference in postprandial TAG response after the two interventions. This may involve an interaction between the high-carbohydrate diet and the carbohydrate content of the test meal. As mentioned in section 2.6.1, it has been demonstrated that carbohydrate (fructose and sucrose) added to a fat load augments postprandial lipaemia compared to a fat-only meal (Cohen and Schall 1988; Jeppesen et al. 1995). This effect of acute carbohydrate ingestion on TAG response has been attributed, at least partly, to an increase in VLDL-TAG secretion by the liver (Cohen and Schall 1988; Jeppesen et al. 1995). One of several mechanisms may be that carbohydrate ingestion accelerates hepatic glycolytic rate, availability of pyruvate and formation of malonyl-CoA, which in turn inhibits hepatic fatty acids oxidation, diverting them
towards TAG synthesis and the secretory pathway (McGarry and Foster 1980; Sidossis et al. 1998). These responses to the carbohydrate in the meal could have been amplified after the high-carbohydrate diet, priming the liver to secrete more VLDL-TAG postprandially – as well as in the fasted state. This, in turn, would increase the total TAG response. The high TAG concentrations during the late postprandial period (Figure 4.1) after the high-carbohydrate diet are consistent with this explanation, as is the low 3-hydroxybutyrate concentrations (implying lower hepatic fatty acid β-oxidation) and the high NEFA concentrations which could have served as a substrate for hepatic TAG synthesis (Figure 4.3).

Plasma NEFA concentrations were higher after the high-fat diet during the early part of the observation period (Figure 4.3). In the postprandial state, the release of NEFA into the circulation is determined by the activity of intracellular hormone sensitive lipase in the adipose tissue and the extent to which lipoprotein lipase-derived fatty acids are not entrapped in tissues (Fielding and Frayn 1998). Therefore the accentuated NEFA response observed after the high-fat diet could reflect an impairment of the normal postprandial suppression of hormone sensitive lipase by insulin and/or a greater "spillover" of fatty acids from the action of lipoprotein lipase.

In the fasted state, glucose homeostasis was well-maintained after the high-fat diet. However, the postprandial plasma glucose response was much greater after the high-fat diet, despite a similar insulin response. This may reflect impaired uptake of glucose by muscle and other tissues due to the greater availability for oxidation of plasma fatty acids (Randle et al. 1963). According to what has been proposed by Randle et al. (1963), high NEFA concentration leads to increased fatty acid oxidation, resulting in inhibition of glycolysis and glucose uptake. This, in turn, leads to an increase in blood glucose concentration.

The high plasma TAG concentrations after the carbohydrate-rich diet may enhance the opportunity for reciprocal transfer of cholesterol and TAG between HDL and TAG-rich lipoproteins (Miesenböck and Patsch 1992), explaining the observation that HDL-cholesterol was lower. However, the lower HDL-cholesterol concentration after a high-carbohydrate diet could also simply reflect lower requirements for HDL-
mediated cholesterol removal at lower intakes of dietary fat (Vélez-Carrasco et al. 1999). It is interesting that the three-day intervention was enough to provoke the expected lowering of HDL-cholesterol by the high-carbohydrate diet. As modulation of HDL-cholesterol is likely to be slower than changes in TAG after the initiation of a carbohydrate-rich diet, this supports the validity of the present dietary intervention model.

In conclusion, the short-term consumption of a high-carbohydrate diet reduced plasma fasting HDL-cholesterol, increased fasting TAG concentrations and elevated the postprandial TAG response to a standard meal, compared with an isoenergetic high-fat diet. These findings complement earlier studies (Jeppesen et al. 1997; Chen et al. 1993; Coulston et al. 1983), which employed test meals with the same macronutrient composition as the preceding diet interventions. However, in order to provoke clear metabolic changes, extreme dietary interventions were employed in the present study. A high-carbohydrate diet was employed in which 70% of energy came from carbohydrate, a more extreme dietary change than that recommended for the population at large. The high-fat diet was very high in fat and associated with metabolic changes observed in a state of insulin resistance, i.e. higher plasma glucose and NEFA concentrations. For these reasons, the present findings should not be interpreted as providing guidance on dietary recommendations.

The potential atherogenicity of carbohydrate-induced hypertriacylglycerolemia and concomitant decreases to HDL-cholesterol are the subject of current debate (Katan et al. 1997; Bierman 1995; Connor and Connor 1997; Ornish 1998; Connor and Connor 1998; Katan et al. 1998). Most of these discussions have so far been based on observations of fasting plasma lipids. However, postprandial plasma TAG concentration in the late postprandial phase (6 hours after fat intake) constitutes an independent risk marker for CHD (Patsch et al. 1992). Interestingly, augmented late postprandial TAG concentrations were observed after the carbohydrate-rich diet. It is not known, however, whether or not this carbohydrate-induced postprandial elevation of TAG is likely to confer a level of atherogenic risk similar to that evident in individuals consuming diets higher in fat.
In the present study, two extreme diet interventions were employed, with regard to their carbohydrate and fat contents. Thus, this study did not provide any information on the magnitude of postprandial lipaemia after a diet of typical Western composition in comparison to after the high-carbohydrate diet. Data from other studies (Chen et al. 1993; Jeppesen et al. 1997) which have used diets of less extreme composition than those in the present study suggest that postprandial lipaemia is higher after a 60 % carbohydrate diet than after a 40 % carbohydrate diet, the latter diet being fairly close to a typical Western diet (46 % carbohydrate). Another important issue in relation to CHD risk is whether carbohydrate-induced hypertriacylglycerolaemia can be avoided. As discussed in section 2.9.2, exercise even of moderate intensity (Aldred et al. 1994; Tsetsonis et al. 1997; Gill and Hardman 2000) beneficially affects postprandial TAG metabolism, at least when subjects are on their “normal” diet. Hypothetically, daily exercise during a high-carbohydrate diet could offset the effects of this diet on TAG metabolism. Thus, a study examining the effect of exercise on postprandial lipaemia, as one means to prevent carbohydrate-induced hypertriacylglycerolaemia, is considered in the chapter that follows.
CHAPTER 5
EXERCISE PREVENTS THE AUGMENTATION OF POSTPRANDIAL LIPAEMIA ATTRIBUTABLE TO A LOW-FAT, HIGH-CARBOHYDRATE DIET

5.1 Introduction
A number of studies (Coulston et al. 1983; Chen et al. 1993; Chen et al. 1995; Jep­pesen et al. 1997) have demonstrated that high-carbohydrate diets (55-60 % carbohydrate) augment postprandial lipaemia compared to diets lower in carbohydrate (40 % carbohydrate). However, in all postprandial studies just referred to, the test meal employed after the interventions was not standardised with regard to its macronutrient composition. The findings of the previous study (chapter 4) complemented those of the above-mentioned studies by demonstrating that the augmentation of postprandial lipaemia after a high-carbohydrate is due to the effect of this diet per se and maybe also its interaction with the macronutrients in the standard test meal.

A sluggish postprandial TAG metabolism constitutes an independent risk marker for CHD (Patsch et al. 1992; Karpe et al. 1998). In addition, the magnitude of postprandial lipaemia could be a determinant of the population of small dense LDL particles (Karpe et al. 1993) and of HDL-cholesterol levels (Lechleitner et al. 1990). It is, therefore, important that the effects of high-carbohydrate diets are investigated in the postprandial state and that ways are found to introduce such diets without adversely affecting plasma TAG concentrations. An increase in physical activity may be effective in this regard, as it has been shown to reduce postprandial lipaemia, at least when subjects are on “normal” Western diets (Aldred et al. 1994; Tsetsonis et al. 1997; Gill and Hardman 2000). It may be therefore that the simultaneous adoption of a low-fat, high-carbohydrate diet and a physically active lifestyle can offset the potentially deleterious effects of this diet on TAG metabolism and optimise, in this way, effects on lipoprotein lipid markers with regard to CHD risk.

A small number of studies in human volunteers have looked at the effect of exercise as one means to prevent carbohydrate-induced hypertriacylglycerolaemia (Liebman
et al. 1983; Ullrich and Albrink 1986; Szostak and Cybulska 1987; Thompson et al. 1988) but, without exception, these studies were undertaken in the fasted state. In the study by Liebman et al. (1983), exercising and sedentary groups were requested to consume their usual diets with the exception that isoenergetic amount of either white bread or white bread containing wheat bran was substituted for their normal bread consumption and/or incorporated into at least two meals per day for 12 weeks (on average, four to six slices of bread were incorporated into the subjects’ diets). Subjects in the exercise groups participated in a 6.4-km (4-mile) walk-jog-run programme at moderate intensity three times per week. Subjects in the sedentary group maintained their normal physical activity levels. The dietary intervention resulted in an isoenergetic substitution of carbohydrate for fat. Specifically, the percentage of daily energy intake derived from carbohydrate increased from 46% before the intervention to 53% in week 10 of the intervention. Corresponding values for fat intake were 38% and 31%. In sedentary and exercise groups, total and VLDL-TAG concentrations increased by week 6 of the intervention and, in the sedentary groups, they continued to rise between week 6 and 12. In contrast, in the exercise groups, the increases in total and VLDL-TAG concentrations observed by week 6 were reversed between week 6 and 12. This last observation was attributed to the exercise regimen as well as the 1.2 kg reduction in mean body weight that occurred during this time period. In a subsequent cross-sectional study (Ullrich and Albrink 1986), subjects followed an euenergetic 70% carbohydrate diet for seven days while either refraining from exercise (non-exercisers) or performing at least 11.3 km (7 miles) over these days. Although both groups exhibited a significant increase in plasma TAG concentration following the high-carbohydrate diet, the increase was significantly lower for the exercisers (0.36 mmol/L) than for the non-exercisers (0.84 mmol/L). Thus, although baseline TAG concentration was not different between the groups, following the high-carbohydrate diet, the non-exercisers had significantly higher fasting TAG concentration (2.17 mmol/L) than the exercisers (1.40 mmol/L). Szostak and Cybulska (1987) also observed that physical exercise, 45 minutes of gymnastic exercises and 15 minutes of swimming three times in a week, reduced or entirely abolished the hypertriacylglycerolaemic effect of a seven-day high fructose intake (80 g per day) in patients with endogenous hypertriacylglycerolaemia. Finally, Thompson et al. (1988) observed that increases in
plasma TAG concentration on a 56 % carbohydrate diet were blunted by physical activity.

To the author's knowledge, there is no information on the effect of exercise during a high-carbohydrate diet on postprandial lipaemia. The present study was designed to test the hypothesis that daily moderate intensity exercise offsets the augmentation of postprandial plasma TAG response associated with a high-carbohydrate diet. A short-term dietary intervention model was employed, in an attempt to investigate whether exercise can prevent the development of hypertriacylglycerolaemia during the initial, dynamic period of change to a high-carbohydrate diet. Exercise was of moderate intensity for 30 minutes daily, as recommended by the Centers for Disease Control and Prevention and the American College of Sports Medicine (Pate et al. 1995).

5.2 Subjects and methods

5.2.1 Subjects

Nine normolipidaemic men took part in the study, which was approved by the Loughborough University Ethical Advisory Committee. All subjects were fully informed of the procedures and risks involved and gave their written consent. Some physical characteristics and plasma concentrations of TAG, total cholesterol and HDL-cholesterol of subjects as measured after an over-night fast on recruitment, whilst subjects were following their habitual lifestyle are presented in Table 5.1. All subjects were non-smokers and none had any physician-diagnosed cardiovascular or metabolic disease or was taking drugs known to influence lipid or carbohydrate metabolism. To exclude individuals with extreme dietary practices, only non-vegetarian subjects and subjects with habitual fat intakes constituting between 25 % and 45 % of energy intake were accepted. Subjects' habitual diet was assessed by the weighed food inventory method over two weekdays and one weekend day, as described in section 3.2.1. Information about the energy content and macronutrient composition of subjects' habitual diets is presented in Table 5.2. All subjects were recreationally active in moderate intensity exercise (e.g. fast walking, easy cycling) at least three times per week. Four of them also engaged in more strenuous exercise,
again around three times per week. Four subjects possessed the E4/3 phenotype, three the E3/3, one the E3/2 and one the E4/4.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, year</td>
<td>33 ± 4</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>79.0 ± 6.6</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.79 ± 0.06</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.4 ± 1.2</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>85.4 ± 4.6</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.86 ± 0.05</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>16.6 ± 1.9</td>
</tr>
<tr>
<td>VO₂ max, mL/kg/min</td>
<td>54 ± 7</td>
</tr>
<tr>
<td>TAG, mmol/L</td>
<td>1.10 ± 0.47</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.49 ± 1.02</td>
</tr>
<tr>
<td>HDL-cholesterol, mmol/L</td>
<td>1.17 ± 0.18</td>
</tr>
</tbody>
</table>

Table 5.1 Physical characteristics of subjects and plasma concentrations of lipoprotein lipids measured in the fasted state. n = 9 men, mean ± SD.
5.2.2 Study design
Each subject consumed a standardised high-fat, mixed meal, with intervals of 13 days, after three different interventions. These interventions comprised: (i) three days on a typical Western diet; (ii) three days on a high-carbohydrate diet; and (iii) three days on the same high-carbohydrate diet with one 30-minute session of moderate intensity exercise each day. The order of the interventions was counterbalanced.

5.2.3 Preliminary exercise tests
Two preliminary exercise tests were conducted. In the first test, the steady-state relationship between submaximal $\dot{V}O_2$ and treadmill speed was established (section 3.2.2.1). From this relationship, a linear regression equation was derived, for each subject, which was used, together with the measured $\dot{V}O_2$ max, to calculate the speed necessary to elicit intensity corresponding to 60 % $\dot{V}O_2$ max. In the second test, each subject’s $\dot{V}O_2$ max was determined during uphill running or walking at a constant speed (section 3.2.2.2 for full details).

5.2.4 Experimental diets
The experimental diets were isoenergetic and their energy value approximated, on an individual basis, subjects’ previously determined energy intake (sections 3.2.1 and 3.8.1). One diet was of similar composition to a typical UK (or Western) diet (Ministry of Agriculture Fisheries and Food 1997). It provided 46 % of energy as carbohydrate, 38 % as fat, and 16 % as protein. The other diet was high in carbohydrate and provided 70 % as carbohydrate, 15 % as fat and 15 % as protein. The diets were based on normal foods (that were provided by the experimenter), excluding alcohol, and their energy and macronutrient contents are shown in Table 5.2. The contributions of saturated, monounsaturated and polyunsaturated fatty acids to the total energy intake in the typical Western diet were designed to equal those for British male adults of 25-34 years of age (Gregory et al. 1990). These contributions were then proportionally reduced for the high-carbohydrate diet. Although the contribution of simple sugars and starch to the total carbohydrate intake for British male adults of 25-34 years is approximately 42 % and 58 %, respectively (Gregory et al. 1990), it was decided that a ratio of 50:50 would enhance the effect of the high-
carbohydrate diet and, therefore, provide a clearer picture of the postprandial TAG responses. Three meals and one or two snacks were consumed each day. Subjects prepared these themselves, to a menu agreed with the experimenter. This included detailed instructions about the mass of each item to be consumed and methods of preparation and cooking (these were discussed verbally as well). Subjects were instructed to follow the experimental diets "to the gram" and were also asked to weigh and record each item consumed. Examples of the prescribed typical Western diet and high-carbohydrate diet are presented in Table 5.3 and Table 5.4, respectively. Subjects refrained from exercise during the three-day interventions, except the planned exercise session in the high-carbohydrate plus exercise intervention. Only activities of daily living and slow walking for personal transport were permitted. Caffeine consumption was not restricted during the interventions but subjects were instructed to standardise it for the day prior the oral fat tolerance tests.

During the three days leading up to the first intervention period, subjects consumed a standardised, prescribed diet (46 %, 38 % and 16 % of energy derived from carbohydrate, fat and protein, respectively) and refrained from alcohol consumption and from exercise. There was a 10-day washout period between interventions, during the first seven days of which subjects resumed their usual physical activity and dietary habits. During the last three days of this period, i.e. leading up to the next intervention, they consumed the same standardised, prescribed diet as prior to the first intervention, again refraining from alcohol consumption and exercise.
Table 5.2  
Energy content and composition of the habitual and experimental (typical Western and high-carbohydrate) diets. Each experimental diet was consumed for three days. Mean or Mean ± SD.

<table>
<thead>
<tr>
<th></th>
<th>Habitual diet</th>
<th>Western diet</th>
<th>High-carbohydrate diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, MJ</td>
<td>10.6 ± 2.0</td>
<td>10.9 ± 1.2</td>
<td>10.9 ± 1.2</td>
</tr>
<tr>
<td>Carbohydrate, %</td>
<td>50 ± 5</td>
<td>46</td>
<td>70</td>
</tr>
<tr>
<td>Sugars</td>
<td>22 ± 5</td>
<td>23</td>
<td>36</td>
</tr>
<tr>
<td>Starch</td>
<td>28 ± 7</td>
<td>23</td>
<td>34</td>
</tr>
<tr>
<td>Fat, %</td>
<td>34 ± 6</td>
<td>38</td>
<td>15</td>
</tr>
<tr>
<td>Saturated</td>
<td>13 ± 4</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>12 ± 5</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>8 ± 4</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>Cholesterol, mg</td>
<td>298 ± 33</td>
<td>260 ± 29</td>
<td>159 ± 25</td>
</tr>
<tr>
<td>Protein, %</td>
<td>16 ± 2</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Fibre, g</td>
<td>22 ± 11</td>
<td>12 ± 1</td>
<td>12 ± 1</td>
</tr>
</tbody>
</table>
Typical Western diet
Total energy: 12.0 MJ
CHO : FAT : PROTEIN
46% : 38% : 16%

<table>
<thead>
<tr>
<th>Breakfast</th>
<th>Amount g</th>
<th>Actual amount, g</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>White bread</td>
<td>92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jam (not reduced sugar)</td>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-fat yogurt, fruit (~1g fat/100g)</td>
<td>180</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coffee with semi-skimmed milk</td>
<td>46 (milk)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sugar</td>
<td>10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Lunch

| White bread                      | 139      |                  |                |
| Flora, 38% vegetable fat         | 32       |                  |                |
| Ham                             | 104      |                  |                |
| Cheddar cheese, medium           | 30       |                  |                |
| Cucumber, lettuce                | Free     |                  |                |
| Orange juice, unsweetened        | 231      |                  |                |

Snack

| Rice pudding, canned (not light)| 231      |                  |                |
| Apple (weighed with core)        | 144      |                  |                |

Dinner

| White spaghetti, boiled          | 277      |                  |                |
| Home-made sauce:                 |          |                  |                |
| Tomatoes, carrots, peppers      | Free     |                  |                |
| Sunflower oil                   | 28       |                  |                |
| Beef mince, not lean (for sauce)| 139 (raw)|                  |                |
| Cheddar cheese, medium           | 28       |                  |                |

Snack

| Orange juice, unsweetened       | 231      |                  |                |
| Apple (weighed with core)       | 144      |                  |                |

Please eat and drink everything that has been prescribed for the three days of the typical Western diet. Please refrain from exercise on these three days and do not eat or drink anything else (except water).

Table 5.3 An example of the prescribed typical Western diet. It was consumed for three days.
### Table 5.4

An example of the prescribed high-carbohydrate diet. It was consumed for three days.

<table>
<thead>
<tr>
<th>Breakfast</th>
<th>Amount, g</th>
<th>Actual amount, g</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn flakes</td>
<td>61</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Semi-skimmed milk</td>
<td>319</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sugar</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raisins</td>
<td>37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple (weighed with core)</td>
<td>153</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White bread</td>
<td>98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Honey (or jam-not reduced sugar)</td>
<td>45 (49)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Snack</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Banana (weighed flesh only)</td>
<td>123</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raisins</td>
<td>49</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lunch</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White bread</td>
<td>147</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ham</td>
<td>74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cucumber, lettuce</td>
<td>Free</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-fat yogurt, fruit (~1g fat/100g)</td>
<td>184</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Banana (weighed flesh only)</td>
<td>123</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange juice, unsweetened</td>
<td>200</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dinner</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White rice, easy cook, boiled</td>
<td>294</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweetcorn, no sugar/salt added</td>
<td>123</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean turkey breast, fried</td>
<td>147 (raw)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn oil</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange juice, unsweetened</td>
<td>241</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5.2.5 Exercise sessions
During the high-carbohydrate diet plus exercise intervention, subjects ran or walked (1 subject) on the treadmill at 60% $\dot{V}O_2$ max for 30 minutes daily. As discussed in section 3.8.2, exercise at this intensity is considered as moderate by most classifications. All exercise sessions were performed in the laboratory between 13:00-17:00 hours. Expired air samples were collected every 15 minutes using Douglas bags and $\dot{V}O_2$, and $\dot{V}CO_2$ were calculated as described in section 3.4. Heart rate was monitored using short-range telemetry (section 3.5) and ratings of perceived exertion using the Borg scale (section 3.6).

5.2.6 Test meal protocol
Full details of the test meal protocol were given in section 3.9. Briefly, subjects arrived at the laboratory after a 12-hour fast at approximately 08:00 hours. A cannula was introduced into a forearm or antecubital vein and the subject rested quietly in a supine position for 10 minutes, after which a baseline blood sample was obtained (details on blood sampling are given in section 3.10). The test meal (described in section 3.9.1) was then consumed. It was given according to body mass (per kg of body mass: 1.2 g fat, 1.1 g carbohydrate, 0.2 g protein). For these subjects this meant (mean ± SD) 95 ± 8 g fat, 86 ± 7 g carbohydrate, 14 ± 1 g protein and 5.14 ± 0.14 MJ of energy, 69% of which came from fat. Further blood samples were obtained (with subjects in the supine position) 15, 30, 45, 60 and 90 minutes after completion of the meal, and then hourly until 6 hours. Expired air samples were collected for 6-minute periods in the fasted state and postprandially (every hour), using Douglas bag techniques, for measurement of $\dot{V}O_2$ and $\dot{V}CO_2$ (section 3.4). Subjects rested or worked quietly during the observation period and consumed only water. This was provided ad libitum on the first trial and replicated during subsequent trials.
5.2.7 Diet analysis
Weighed food inventories were analysed for energy and major nutrients using a computerised version (Comp-Eat 5.0, Nutrition Systems, London) of UK food composition tables (Holland et al. 1991).

5.2.8 Analytical methods
At each sampling point in the fasted and postprandial states, blood samples for plasma preparation were separated within 15 minutes of collection, divided into aliquots and were stored at -20°C. At most time points, separate samples were collected for serum preparation. These were allowed to clot at room temperature before separation and were stored at -70°C. Plasma samples were analysed for total and HDL-cholesterol (fasted and 6-h samples only), TAG, non-esterified fatty acids (NEFA), glucose and lactate, and serum samples for 3-hydroxybutyrate (all by enzymatic, colorimetric methods). Serum samples were also analysed for insulin using a solid-phase 125I radioimmun assay. Details of these analyses have been given in section 3.11. Phenotypes of apolipoprotein E were determined by isoelectric focusing, using Western blot techniques.

5.2.9 Calculations and statistics
Plasma and serum concentrations measured in the fasted state were compared using one-way ANOVA for repeated measures. Total postprandial responses were calculated as the AUC using the trapezoidal rule. These summary measures (section 3.12) were compared among interventions using one-way ANOVA for repeated measures. In addition, the interaction effect of intervention and time was determined using two-way ANOVA for repeated measures. As TAG concentrations and AUC for TAG were not normally distributed, logarithmic transformation was performed before statistical testing (section 3.12). If significant differences were identified by ANOVA, Tukey post hoc comparisons were performed to determine where these differences occurred. Carbohydrate and fat utilisation rates and energy expenditure were calculated using indirect calorimetry, as described in section 3.3. LDL cholesterol concentration in the fasted state was estimated using the Friedewald formula (Friedewald et al. 1972). A 5% level of significance was adopted throughout. Sta-
Statistical procedures were performed using Statistica for Windows, version 5.0 (Tulsa, OK, USA).

5.3 Results
5.3.1 Experimental diets and test meal
Both experimental diets were well tolerated, aside from a common complaint by the subjects of feeling excessively full during the high-carbohydrate diet. Compliance, assessed by food inventories and detailed discussions with subjects, was high. The test meal was also well tolerated by all subjects without any sign of nausea or other gastrointestinal discomfort. It was consumed over a median of 11 (range 9 – 14) minutes.

5.3.2 Cardiorespiratory and metabolic responses during exercise sessions
The average \( \dot{V}O_2 \) during exercise for the 3 days was 33 ± 5 mL/kg/min (mean ± SD) and represented 61 ± 3 % of \( \dot{V}O_2 \) max. This was achieved by running on the flat at 2.6 ± 0.4 m/s (8 subjects). One subject walked at 2.1 m/s up a 4 % gradient. Average values for heart rate and respiratory exchange ratio were 145 ± 19 beat/min and 0.94 ± 0.03 respectively. Ratings of perceived exertion were 12 ± 1 corresponding to the description “fairly hard”. The gross energy expenditure was 1.58 ± 0.36 MJ, 81 ± 10 % from carbohydrate and 19 ± 10 % from fat. On average, 60 ± 35 g of carbohydrate and 7 ± 4 g of fat were oxidized daily during treadmill running / walking.

5.3.3 Plasma and serum concentrations in the fasted state
Fasting plasma concentrations of TAG, total, HDL- and LDL-cholesterol, NEFA, glucose, lactate and serum concentrations of insulin and 3-hydroxybutyrate are presented in Table 5.5. Triacylglycerol concentration was significantly higher after the high-carbohydrate diet than after the Western diet (\( P = 0.03 \)). The addition of exercise to the high-carbohydrate diet did not significantly reduce TAG concentration (\( P = 0.19 \) vs high-carbohydrate diet). LDL-cholesterol concentrations were significantly lower after both the high-carbohydrate and the high-carbohydrate plus exercise interventions than after the Western diet. There were no significant differences among interventions in any of the other parameters.
<table>
<thead>
<tr>
<th></th>
<th>Western</th>
<th>High-CHO</th>
<th>High-CHO-Ex</th>
<th>P value$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAG, mmol/L</td>
<td>0.83 ± 0.10</td>
<td>1.15 ± 0.16$^1$</td>
<td>1.02 ± 0.24</td>
<td>0.03</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.15 ± 0.27</td>
<td>4.04 ± 0.27</td>
<td>3.95 ± 0.26</td>
<td>0.08</td>
</tr>
<tr>
<td>HDL-cholesterol, mmol/L</td>
<td>1.05 ± 0.08</td>
<td>0.95 ± 0.04</td>
<td>0.98 ± 0.06</td>
<td>0.28</td>
</tr>
<tr>
<td>LDL-cholesterol, mmol/L</td>
<td>2.75 ± 0.25</td>
<td>2.56 ± 0.2$^5$</td>
<td>2.54 ± 0.25$^6$</td>
<td>0.01</td>
</tr>
<tr>
<td>Non-esterified fatty acids, mmol/L</td>
<td>0.26 ± 0.04</td>
<td>0.24 ± 0.05</td>
<td>0.31 ± 0.04</td>
<td>0.37</td>
</tr>
<tr>
<td>3-Hydroxybutyrate, mmol/L</td>
<td>0.04 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.86</td>
</tr>
<tr>
<td>Insulin, μIU/ml</td>
<td>8.1 ± 0.8</td>
<td>8.4 ± 1.3</td>
<td>7.7 ± 0.9</td>
<td>0.74</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.35 ± 0.17</td>
<td>5.29 ± 0.16</td>
<td>5.22 ± 0.13</td>
<td>0.17</td>
</tr>
<tr>
<td>Lactate, mmol/L</td>
<td>0.76 ± 0.07</td>
<td>0.91 ± 0.10</td>
<td>0.81 ± 0.06</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Table 5.5 Plasma or serum concentrations measured in the fasted state after three days on a typical Western diet (Western), after three days on a high-carbohydrate diet (High-CHO) and after three days on the same high-carbohydrate diet with the addition of 30 minutes daily moderate intensity exercise (High-CHO-Ex). Each diet was consumed for three days. n = 9 men, Mean ± SEM.

$^1$ Significance of differences among interventions by one-way ANOVA for repeated measures.

$^5$ Significantly different from Western diet (Tukey post hoc test): $P < 0.05$. 
5.3.4 Postprandial responses

Plasma TAG concentrations after the test meal are shown in Figure 5.1, with summary measures (AUCs) of these responses in Table 5.6. The AUC was 35% greater after the high-carbohydrate diet than after the Western diet ($P < 0.01$). The addition of daily exercise to the high-carbohydrate diet significantly reduced this response, almost to the level observed after the Western diet ($P = 0.01$ vs high-carbohydrate diet). The pattern of change over time in TAG concentration differed among interventions (interaction $P < 0.01$). Peak concentration occurred later after the high-carbohydrate diet, both with and without exercise (5 ± 1 h for both interventions) (mean ± SEM), than after the Western diet (3 ± 1 h, $P < 0.01$).

Postprandial concentrations of insulin, NEFA and 3-hydroxybutyrate, glucose and lactate are shown in Figure 5.2, 5.3 and 5.4 (respectively) and summary measures (AUCs) are given in Table 5.6. None of the AUCs differed among interventions, except 3-hydroxybutyrate. The change in plasma concentrations of glucose over time differed among interventions (interaction of intervention × time, $P < 0.01$). The AUC for 3-hydroxybutyrate was significantly lower after the high-carbohydrate diet than after the Western diet, with a much smaller rise between two and six hours postprandially (interaction of intervention × time, $P < 0.01$). The addition of exercise to the high-carbohydrate diet did not have a significant effect on the AUC for 3-hydroxybutyrate. Neither total nor HDL-cholesterol changed over the postprandial period in any of the interventions.
Figure 5.1  Plasma TAG concentrations in the fasted state (0 hour) and for six hours following consumption of a high-fat mixed meal after three days on a typical Western diet (Western), after three days on a high-carbohydrate diet (High-CHO) and after three days on the same high-carbohydrate diet with 30 minutes daily of moderate exercise (High-CHO-Ex). $n = 9$ men. Mean ± SEM.
Figure 5.2  Serum insulin concentrations in the fasted state (0 hour) and for six hours following consumption of a high-fat mixed meal after three days on a typical Western diet (Western), after three days on a high-carbohydrate diet (High-CHO) and after three days on the same high-carbohydrate diet with 30 minutes daily of moderate exercise (High-CHO-Ex). \( n = 9 \) men. Mean ± SEM.
Figure 5.3  Plasma non-esterified fatty acids (NEFA) and serum 3-hydroxybutyrate concentrations in the fasted state (0 hour) and for six hours following consumption of a high-fat mixed meal after three days on a typical Western diet (Western), after three days on a high-carbohydrate diet (High-CHO) and after three days on the same high-carbohydrate diet with 30 minutes daily of moderate exercise (High-CHO-Ex). n = 9 men. Mean ± SEM.
Figure 5.4  Plasma glucose and lactate concentrations in the fasted state (0 hour) and for six hours following consumption of a high-fat mixed meal after three days on a typical Western diet (Western), after three days on a high-carbohydrate diet (High-CHO) and after three days on the same high-carbohydrate diet with 30 minutes daily of moderate exercise (High-CHO-Ex). n = 9 men. Mean ± SEM.
Table 5.6 Six-hour areas under the plasma or serum concentration vs time curves for TAG, non-esterified fatty acids, 3-hydroxybutyrate, insulin, glucose and lactate after three days on a typical Western diet (Western), after three days on a high-carbohydrate diet (High-CHO) and after three days on the same high-carbohydrate diet with 30 minutes daily of moderate exercise (High-CHO-Ex). \( n = 9 \) men. Mean ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>Western</th>
<th>High-CHO</th>
<th>High-CHO-Ex</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAG, mmol/L × h</td>
<td>9.30 ± 1.30</td>
<td>12.54 ± 2.07( ^{\delta} )</td>
<td>9.95 ± 1.94( ^{\dagger} )</td>
<td>0.004</td>
</tr>
<tr>
<td>Non-esterified fatty acids, mmol/L × h</td>
<td>2.07 ± 0.18</td>
<td>1.74 ± 0.17</td>
<td>1.97 ± 0.15</td>
<td>0.20</td>
</tr>
<tr>
<td>3-Hydroxybutyrate, mmol/L × h</td>
<td>0.42 ± 0.07</td>
<td>0.26 ± 0.07( ^{\dagger} )</td>
<td>0.31 ± 0.07</td>
<td>0.004</td>
</tr>
<tr>
<td>Insulin, μIU/mL × h</td>
<td>126 ± 15</td>
<td>135 ± 16</td>
<td>116 ± 16</td>
<td>0.18</td>
</tr>
<tr>
<td>Glucose, mmol/L × h</td>
<td>34.1 ± 0.9</td>
<td>33.2 ± 1.0</td>
<td>33.4 ± 1.1</td>
<td>0.28</td>
</tr>
<tr>
<td>Lactate, mmol/L × h</td>
<td>5.51 ± 0.26</td>
<td>5.78 ± 0.45</td>
<td>5.70 ± 0.44</td>
<td>0.67</td>
</tr>
</tbody>
</table>

\( ^{\dagger} \) Significance of differences among interventions by one-way ANOVA for repeated measures.

\( ^{\delta, \dagger} \) Significantly different from Western diet (Tukey post hoc test): \( ^{\delta} P < 0.01, ^{\dagger} P < 0.05. \)

\( ^{\dagger} \) Significantly different from High-CHO diet (Tukey post hoc test): \( P < 0.05. \)
5.3.5 Indirect calorimetry

Fasting and postprandial respiratory exchange ratio (RER) values are presented in Figure 5.5. Neither the high-carbohydrate intervention, nor the high-carbohydrate plus exercise intervention, significantly affected fasting or postprandial energy expenditure or substrate utilisation. More details are given below.

In the fasted state, respiratory exchange ratio values were numerically lower after the Western intervention (0.78 ± 0.01) than after both the High-CHO (0.83 ± 0.03) and the High-CHO-Ex (0.80 ± 0.02) interventions but they were not significantly different. Calculated rates of fat utilisation were 0.09 ± 0.01 g/min (Western), 0.08 ± 0.01 g/min (High-CHO) and 0.09 ± 0.01 g/min (High-CHO-Ex) and were not significantly different among interventions. The same was the case for rates of carbohydrate utilisation, which were 0.10 ± 0.03 g/min (Western), 0.15 ± 0.04 g/min (High-CHO) and 0.12 ± 0.03 g/min (High-CHO-Ex). Therefore, calculated resting metabolic rate did not differ significantly among interventions: 5.18 ± 0.22 kJ/min (Western), 5.51 ± 0.38 kJ/min (High-CHO) and 5.34 ± 0.26 kJ/min (High-CHO-Ex).

In the postprandial state, calculated energy expenditure over the 6-hour observation period was not significantly different among interventions: 2.15 ± 0.08 MJ (Western), 2.09 ± 0.10 MJ (High-CHO) and 2.06 ± 0.08 MJ (High-CHO-Ex). The amount of fat utilised during the 6-h postprandial period was 37 ± 1 g (Western), 33 ± 2 g (High-CHO) and 36 ± 1 g (High-CHO-Ex) (NS). Corresponding values for the amount of carbohydrate utilised postprandially were 41 ± 5 g, 53 ± 8 g and 44 ± 5 g (NS).
Figure 5.5 Respiratory exchange ratio (RER) values in the fasted state (0 hour) and for six hours following consumption of a high-fat mixed meal after three days on a typical Western diet (Western), after three days on a high-carbohydrate diet (High-CHO) and after three days on the same high-carbohydrate diet with 30 minutes daily of moderate exercise (High-CHO-Ex). $n = 9$ men. Mean $\pm$ SEM.
5.4 Discussion
The main purpose of the present study was to investigate whether the addition of daily moderate intensity exercise can oppose the exaggeration in postprandial plasma TAG response attributable to a high-carbohydrate diet. The exaggeration in postprandial lipaemia was examined by comparing the postprandial TAG response after a high-carbohydrate diet with that after a typical Western diet.

As expected (Chen et al. 1993; Jeppesen et al. 1997), the high-carbohydrate diet augmented postprandial lipaemia compared to the Western diet. However, in line with the hypothesis in the present study, this carbohydrate-induced increase was negated by 30 minutes of daily moderate intensity exercise. Previous studies have shown that such exercise reduces postprandial lipaemia (Aldred et al. 1994; Tsetsonis et al. 1997; Gill and Hardman 2000) but, without exception, these studies were undertaken with the subjects' background diets ('normal' Western diets) held constant. These are the first data to demonstrate that the TAG-lowering effect of moderate exercise is sufficiently potent to offset the augmentation of postprandial lipaemia which develops when subjects change from a typical Western diet to a low-fat, high-carbohydrate diet.

Other studies have shown that physical activity can ameliorate fasting hypertriacylglycerolaemia associated with a high-carbohydrate diet (Liebman et al. 1983; Ullrich and Albrink 1986; Szostak and Cybulski 1987; Thompson et al. 1988). Interestingly, in the present study, there was no significant effect on fasting TAG concentrations of adding exercise to the high-carbohydrate diet. By contrast, when the TAG metabolic capacity was challenged in the postprandial state the normalising effect of exercise was clearly revealed, showing the importance of studying humans in the fed state.

There are a number of possible explanations for the carbohydrate-induced exaggeration in postprandial lipaemia. Whilst the higher plasma TAG concentrations observed in the fasted state after the high-carbohydrate diet would have contributed (Bruntell et al. 1973; Karpe and Hultin 1995; Björkegren et al. 1996), the high-carbohydrate diet probably also affected postprandial events per se. As discussed in
detail in chapter 4, although the same test meal was consumed on each occasion, the
effect on lipaemia of the carbohydrate in the meal could have been magnified after
the high-carbohydrate diet. Carbohydrate in a meal accentuates fat-induced post­
prandial lipaemia, possibly due to stimulation of endogenous TAG secretion (Cohen
and Schall 1988; Jeppesen et al. 1995). High-carbohydrate diets prime the liver to
secrete more VLDL-TAG, at least partly, by suppressing hepatic oxidation of fatty
acids and so diverting a greater proportion of them towards hepatic TAG synthesis
(Sidossis and Mittenddorfer 1999; McGarry and Foster 1980). It may be therefore
that the response to the carbohydrate in the meal was amplified after the high­
carbohydrate diet, priming the liver to secrete more VLDL-TAG postprandially.
The lower postprandial 3-hydroxybutyrate response after the high-carbohydrate diet
(Table 5.4, Figure 5.3) than after the typical Western diet may indicate a suppres­
sive effect of the former diet on hepatic fatty acid oxidation.

An additional explanation for the augmented postprandial lipemia after the high­
carbohydrate diet may be an effect of this diet on the activity of LPL. For example,
a 56 % reduction in skeletal muscle LPL activity has been reported in sedentary
men who consumed a high-carbohydrate diet for three days (Lithell et al. 1982).
Furthermore, at a whole-body level, a reduction in (heparin releasable) LPL activity
and capacity for clearance of TAG has often been observed after high-carbohydrate
diets (Thompson et al. 1984; Parks et al. 1999). The above alterations would lead to
increases in plasma TAG concentrations, especially in the postprandial state, when
the number of circulating TAG-rich lipoproteins increases dramatically.

The addition of exercise to the high-carbohydrate diet prevented the exaggeration in
plasma postprandial TAG response that was observed with the high-carbohydrate
diet alone. Although there were no significant differences in the fasted state, it may
be that the moderate reductions in fasting TAG concentrations observed in the ma­
majority of subjects contributed to lower responses during the postprandial state. Exer­
cise could also have opposed the suppressive effects of high-carbohydrate diets on
LPL activity that often occur with this type of diet (Lithell et al. 1982; Thompson et
al. 1984). Short-term exercise increases muscle LPL activity (Seip et al. 1997), an
effect opposite to that seen after a short-term high-carbohydrate diet (Lithell et al.
Furthermore, both a single exercise session (Sady et al. 1986) and exercise training (Thompson et al. 1988) increase (heparin releasable) LPL activity and the capacity for intravenous fat clearance. All these exercise-induced changes may have affected postprandial TAG metabolism, when exercise was combined with the high-carbohydrate diet.

Exercise may have also ameliorated the carbohydrate-induced augmentation in postprandial lipemia by affecting VLDL-TAG production. It has been reported that both high-carbohydrate diets (Sidossis and Mittendorfer 1999) and increased acute glucose availability to the liver (Sidossis et al. 1998) increase VLDL-TAG secretion, possibly by increasing hepatic glycolytic rate and therefore inhibiting hepatic fatty acid oxidation. If this were the case during the high-carbohydrate diet, one might speculate that the exercise diverted a considerable amount of carbohydrate to muscles for use as an energy source and for glycogen replenishment. This would reduce the amount of carbohydrate that needed to be disposed of by the liver. This, in turn, might have attenuated the increase in hepatic glycolytic rate and the decrease in hepatic fatty acid oxidation due to the high-carbohydrate diet, decreasing VLDL-TAG secretion. In line with this thinking, the reduction in the postprandial 3-hydroxybutyrate response by the high-carbohydrate diet was somewhat attenuated by exercise, although this difference was not significant.

As expected (Mensink and Katan 1992), the low-carbohydrate diet reduced LDL-cholesterol concentration, although the addition of exercise did not reduce this further. Interestingly, the interventions employed did not alter HDL-cholesterol, despite the fact that both low-carbohydrate diets (Katan 1998) and exercise (Durstine and Haskell 1994) have been reported to influence this. HDL-cholesterol concentrations are dependent on the rate of transfer to HDL of surface lipids from chylomicron and VLDL remnants formed during lipolysis (Patsch et al. 1987). It may be, therefore, that the initial effect of a high-carbohydrate diet or of exercise is to alter postprandial TG metabolism and that changes in HDL-cholesterol concentration develop later.
The present model can not show whether the prevention of carbohydrate-induced augmentation in postprandial lipaemia was due to the effect of the last session of exercise *per se* or to the accumulated effects of the three exercise sessions, or both. However, the first possibility seems unlikely because exercise of longer duration and greater energy expenditure did not significantly decrease postprandial lipaemia (Tsetsonis and Hardman 1996b).

A case has recently been made out for replacing saturated fat in the diet with mono-unsaturated fatty acids as a strategy to reduce the risk of coronary heart disease (Katan 1997). This would reduce LDL-cholesterol without the adverse effects on TAG and HDL-cholesterol concentrations seen when fat is replaced with carbohydrate. The present findings show that a further attractive alternative may be the adoption of a physically active lifestyle *alongside* a low-fat, high-carbohydrate diet. However, it should be noted that a short-term intervention model was employed in order to examine the influence of exercise on the development of hypertriacylglycerolaemia during the initial period of a change to a high-carbohydrate diet. There is no evidence, therefore, on the long-term efficacy of exercise in this regard but this is an issue which justifies further investigation.

In conclusion, daily moderate intensity exercise prevented the augmentation of postprandial lipaemia resulting from the short-term consumption of a low-fat, high-carbohydrate diet. Moreover, a diet was employed in which 70% of energy came from carbohydrate, a more extreme dietary change than that recommended for the population at large. Nonetheless, a modest amount of exercise, attainable by most people, was sufficient to negate carbohydrate-induced hypertriacylglycerolaemia.

Since exercise seems to have such a potent effect on carbohydrate-induced hypertriacylglycerolaemia, a study examining the qualitative effects on postprandial TAG metabolism of exercise during a low-fat, high-carbohydrate diet is considered in the chapter that follows.
CHAPTER 6
POSTPRANDIAL ACCUMULATION OF TRIACYLGLYCEROL-RICH LIPOPROTEINS AFTER A LOW-FAT, HIGH-CARBOHYDRATE DIET: REVERSAL BY MODERATE EXERCISE

6.1 Introduction
The study described in the previous chapter showed that daily moderate intensity exercise is sufficient to prevent the augmentation of postprandial lipaemia which develops when subjects change from a typical Western diet to a low-fat, high-carbohydrate diet. However, a detailed description of the qualitative changes to postprandial TRL metabolism elicited by high-carbohydrate diets and exercise is lacking.

TRL particles are a heterogeneous group of particles including chylomicrons (containing apo B48), VLDL (containing apo B100) and their remnants (section 2.3). TRL remnants are formed from VLDL and chylomicrons after partial removal of their TG by the action of LPL. The remnants have a reduced TG content but are enriched in cholesterol and have been implicated in the development of atherosclerosis (section 2.7.1). Determination of RLP-cholesterol in serum (section 3.11.6) provides a marker for TRL remnants and may be useful in the assessment of CHD risk. Previous reports showed that CHD patients have increased fasting RLP-cholesterol levels (Nakajima et al. 1993; Devaraj et al. 1998). Fasting RLP-cholesterol also predicted the development of clinical coronary events in patients with CHD independently of other risk factors (Kugiyama et al. 1999) and was associated with common carotid artery intima-media thickness in healthy humans independently of plasma TAG and LDL-cholesterol levels (Karpe et al. in press).

The purpose of the present study was to investigate the effect of a high-carbohydrate diet and exercise on TRL particles of exogenous and endogenous origin. A further purpose was to provide some indirect evidence for the atherogenic potential of carbohydrate-induced changes to TRL metabolism. Postmenopausal women were studied as plasma TAG concentration is an important CHD risk factor for women (Hokanson and Austin 1996) and CHD risk increases after the menopause (Kannel et al. 1976). In addition, despite the popular perception that CHD is a
male disease, it is responsible for 20% of deaths in women, making it the leading cause of female mortality (Office for National Statistics 1998).

6.2 Subjects and methods
6.2.1 Subjects
Eight healthy postmenopausal (for at least two years) women took part in the study, which was approved by the Loughborough University Ethical Advisory Committee. All subjects were fully informed of the procedures and risks involved and gave their written consent. Some physical characteristics and plasma concentrations of TAG, total cholesterol and HDL-cholesterol of subjects as measured after an over-night fast on recruitment, whilst subjects were following their habitual lifestyle are presented in Table 6.1. All subjects were non-smokers and none had any physician-diagnosed cardiovascular or metabolic disease. Two subjects were on hormone replacement therapy and were instructed not to interrupt it during the study. Other than this, subjects were not taking drugs known to influence lipid or carbohydrate metabolism. To exclude individuals with extreme dietary practices, only non-vegetarian subjects and subjects with habitual fat intakes constituting between 25% and 45% of energy intake were enrolled. Subjects' habitual diet was assessed by the weighed food inventory method over two weekdays and one weekend day, as described in section 3.2.1. Information about the energy content and macronutrient composition of subjects' habitual diets is presented in Table 6.2. All subjects engaged in activities like fast walking or easy cycling at least three times per week. Six subjects possessed the E3/3 phenotype, one the E4/3, and one the E4/2.
<table>
<thead>
<tr>
<th>Physical characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, year</td>
<td>60 ± 4</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>69.6 ± 6.6</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.63 ± 0.04</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.4 ± 2.3</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>86.9 ± 4.8</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.84 ± 0.05</td>
</tr>
<tr>
<td>V̇O₂ max, mL/kg/min</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>TAG, mmol/L</td>
<td>1.11 ± 0.31</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.50 ± 0.46</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.69 ± 0.29</td>
</tr>
</tbody>
</table>

Table 6.1  Physical characteristics of subjects and plasma concentrations of lipoprotein lipids measured in the fasted state. n = 8 women, mean ± SD.
6.2.2 Study design
Each subject consumed a standardised high-fat, mixed meal, with intervals of 13 days, after three different interventions. These interventions comprised: (i) three days on a low-carbohydrate diet (Low-CHO); (ii) three days on a high-carbohydrate diet (High-CHO); and (iii) three days on the same high-carbohydrate diet with one 60-minute session of brisk walking each day (High-CHO-Ex). The order of the interventions was counterbalanced.

6.2.3 Preliminary exercise testing
The steady-state relationship between submaximal $\dot{V}O_2$ and treadmill gradient was established during a submaximal uphill walking test at a constant speed (section 3.2.2.1). From this relationship, a linear regression equation was derived, for each subject, which was used, together with the estimated $\dot{V}O_2$ max, to calculate the gradient necessary to elicit intensity corresponding to 60% $\dot{V}O_2$ max. As the subjects were women 51-66 years and had not undertaken any clinical exercise stress test to ensure no contraindications to maximal exercise, their $\dot{V}O_2$ max was estimated, rather than directly measured, by extrapolation of the $\dot{V}O_2$/heart rate relationship obtained during the submaximal exercise test (section 3.2.2.2).

6.2.4 Experimental diets
The experimental diets were isoenergetic and their energy value approximated, on an individual basis, subjects' previously determined energy intake (sections 3.2.1 and 3.8.1). The low-carbohydrate diet provided 35% of energy as carbohydrate, 50% as fat, and 15% as protein. Corresponding values for the isoenergetic high-carbohydrate diet were 70%, 15% and 15%. The diets were based on normal foods (that were provided by the experimenter), excluding alcohol, and their energy and macronutrient contents are shown in Table 6.2. A low-carbohydrate diet, rather than a typical Western diet, was employed in order to enhance the chance of observing clear differences in the postprandial events. For the same reason and in order to exaggerate the effect of the high-carbohydrate diet on TAG metabolism, the ratio of simple sugars to starch in the high-carbohydrate diet employed in the present study (67:33) was much higher than in the high-carbohydrate diet employed in the two previous studies (50:50). Using diets of more extreme composition than those em-
ployed in chapter 5 would increase the chance of detecting diet effects on TRL particles, taking into consideration that analytical imprecision for the TRL determination would be greater than that for simple assays (e.g. plasma TAG concentration). Three meals and one or two snacks were consumed each day. Subjects prepared these themselves, to a menu agreed with a dietician. The menu included detailed instructions about the mass of each item to be consumed and methods of preparation and cooking (these were also discussed verbally with the dietician). Subjects were instructed to follow the experimental diets “to the gram” and were also asked to weigh and record each item consumed. Examples of the prescribed low-carbohydrate diet and high-carbohydrate diet are presented in Table 6.3 and Table 6.4, respectively. Subjects refrain from exercise during the three-day interventions, except the planned exercise session in the High-CHO-Ex intervention. Only activities of daily living and slow walking for personal transport were permitted. Caffeine consumption was not restricted during the interventions but subjects were instructed to standardise it on the day prior to the oral fat tolerance tests.

During the day prior to the first intervention subjects weighed and recorded all food and drink consumed, replicating this prior to subsequent interventions. During this day, they also refrained from exercise and from alcohol consumption. Otherwise, subjects returned to their usual lifestyle during the 10-day wash-out intervals.
<table>
<thead>
<tr>
<th></th>
<th>Habitual</th>
<th>Low-carbohydrate</th>
<th>High-carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy (MJ)</strong></td>
<td>7.41 ± 1.37</td>
<td>7.45 ± 1.39</td>
<td>7.41 ± 1.39</td>
</tr>
<tr>
<td><strong>Carbohydrate, % energy</strong></td>
<td>46 ± 4</td>
<td>35</td>
<td>70</td>
</tr>
<tr>
<td>Simple sugars</td>
<td>21 ± 3</td>
<td>18</td>
<td>47</td>
</tr>
<tr>
<td>Starch</td>
<td>25 ± 4</td>
<td>17</td>
<td>23</td>
</tr>
<tr>
<td><strong>Fat, % energy</strong></td>
<td>37 ± 5</td>
<td>50</td>
<td>15</td>
</tr>
<tr>
<td>Saturated</td>
<td>15 ± 3</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>11 ± 3</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>7 ± 2</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td><strong>Protein, % energy</strong></td>
<td>17 ± 3</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td><strong>Cholesterol, mg</strong></td>
<td>206 ± 33</td>
<td>331 ± 131</td>
<td>131 ± 25</td>
</tr>
<tr>
<td><strong>Fibre, g</strong></td>
<td>19 ± 3</td>
<td>13 ± 3</td>
<td>13 ± 3</td>
</tr>
</tbody>
</table>

Table 6.2 Energy content and composition of the habitual and experimental (low- and high-carbohydrate) diets. Each experimental diet was consumed for three days. Mean or Mean ± SD.
**Low-carbohydrate diet**

Total energy: 8.8 MJ

<table>
<thead>
<tr>
<th>CHO (%)</th>
<th>FAT (%)</th>
<th>PROTEIN (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>35%</td>
<td>50%</td>
<td>15%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Breakfast</th>
<th>Amount, g</th>
<th>Actual amount, g</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wholemeal bread</td>
<td>53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flora, 70% vegetable fat</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole milk</td>
<td>200</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lunch</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Wholemeal bread</td>
<td>80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flora, 70% vegetable fat</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheddar cheese, medium</td>
<td>73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cucumber, lettuce</td>
<td>Free</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Snack</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Walnuts (weighed without shells)</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple (weighed with core)</td>
<td>260</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Banana (weighed flesh only)</td>
<td>75</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dinner</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Quiche Lorraine, Sainsbury’s</td>
<td>168</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oven chips, McCain reduced fat, baked</td>
<td>73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomatoes, carrots, peppers</td>
<td>Free</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-fat yogurt, fruit (~1g fat/100g)</td>
<td>150</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please eat and drink everything that has been prescribed for the three days of the low-carbohydrate diet. Please refrain from exercise on these three days and do not eat or drink anything else (except water).

Table 6.3  An example of the prescribed low-carbohydrate diet. It was consumed for three days.
## High-carbohydrate diet

Total energy: 8.7 MJ

<table>
<thead>
<tr>
<th></th>
<th>Amount, g</th>
<th>Actual amount, g</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Breakfast</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn flakes</td>
<td>36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Semi-skimmed milk</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raisins</td>
<td>42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple (weighed with core)</td>
<td>92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wholemeal bread</td>
<td>36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jam (not reduced sugar)</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lunch</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wholemeal bread</td>
<td>71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean ham</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cucumber, lettuce</td>
<td>Free</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange juice, unsweetened</td>
<td>339</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Banana (weighed flesh only)</td>
<td>240</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Snack</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digestive biscuits (plain)</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Semi-skimmed milk</td>
<td>200</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dinner</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White rice, easy cook, boiled</td>
<td>119</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweetcorn, no sugar/salt added</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean turkey breast, fried</td>
<td>110 (raw)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn oil</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange juice, unsweetened</td>
<td>339</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Snack</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple (weighed with core)</td>
<td>119</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boiled sweets (total for the whole day)</td>
<td>68</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please eat and drink everything that has been prescribed for the three days of the high-carbohydrate diet. Please refrain from exercise on these three days and do not eat or drink anything else (except water).

Table 6.4 An example of the prescribed high-carbohydrate diet. It was consumed for three days.
6.2.5 Exercise sessions
During the High-CHO-Ex intervention, subjects walked on the treadmill at 60 % of estimated \( \dot{V}O_2 \) max for 60 minutes each afternoon. The duration of exercise employed was longer than that in chapter 5 in order to increase the chance of detecting an effect of exercise on TRL particles. However, the intensity remained moderate in order to be attainable by the population at large. Exercise at this intensity for the group of older subjects in the present study meant brisk walking. All exercise sessions were performed in the laboratory between 13:00-17:00 hours. Expired air samples were collected every 15 minutes using Douglas bags and \( \dot{V}O_2 \) and \( \dot{V}CO_2 \) were calculated as described in section 3.4. Heart rate was monitored using short-range telemetry (section 3.5) and ratings of perceived exertion using the Borg scale (section 3.6).

6.2.6 Test meal protocol
Full details of the test meal protocol are given in section 3.9. Briefly, subjects arrived at the laboratory after a 12-hour fast at approximately 08:00 hours. A cannula was introduced into a forearm or antecubital vein and the subject rested quietly in a supine position for 10 minutes, after which a baseline blood sample was obtained (details on blood sampling are given in section 3.10). The test meal (described in section 3.9.1) was then consumed. It was given according to body mass (per kg of body mass: 1.0 g fat, 0.9 g carbohydrate, 0.2 g protein). For these subjects this meant (mean ± SD) 62 ± 6 g fat, 56 ± 6 g carbohydrate, 9 ± 1 g protein and 3.36 ± 0.35 MJ of energy, 69 % of which came from fat. Further blood samples were obtained (with subjects in the supine position) 15, 30, 45, 60 and 90 minutes after completion of the meal, and then hourly until 6 hours. Expired air samples were collected for 6-minute periods in the fasted state and postprandially (every hour), using Douglas bag techniques, for measurement of \( \dot{V}O_2 \) and \( \dot{V}CO_2 \) (section 3.4). Subjects rested or worked quietly during the observation period and consumed only water. This was provided \textit{ad libitum} on the first trial and replicated during subsequent trials.
6.2.7 Diet analysis
Weighed food inventories were analysed using a computerised version (Comp-Eat 5.0, Nutrition Systems, London) of UK food composition tables. (Holland et al. 1991)

6.2.8 Analytical methods
At each sampling point in the fasted and postprandial states, blood samples for plasma preparation were separated within 15 minutes of collection, divided into aliquots and were stored at -20°C. At most time points, separate samples were collected for serum preparation. These were allowed to clot at room temperature before separation and were stored at -70°C. One plasma aliquot was kept overnight on ice, until a TRL fraction was separated by preparative ultracentrifugation. To minimise proteolytic degradation of apo B in this aliquot, 1.0 μL per mL plasma of phenylmethylsulfonylfluoride (PMSF, Sigma) 10 mM dissolved in iso-propanol and 5 μL per mL plasma of aprotinin (Trasylol, Bayer, Leverkusen, Germany) 1.4 μg/L were added. Further plasma aliquots were stored at -20°C until analysed for total cholesterol, HDL-cholesterol (fasted samples only), glucose and NEFA by enzymatic, colorimetric methods (section 3.11.1). Concentrations of TAG in plasma and the TRL fraction were measured enzymatically, with correction for free glycerol, (Humphreys et al. 1990) in samples obtained at all time points (total TAG in frozen samples) or at 0, 2, 4 and 6 hours (TRL-TAG in fresh samples). Serum aliquots were stored at -70°C, until analysed for insulin (section 3.11.2), RLP-cholesterol (section 3.11.6) and 3-hydroxybutyrate (3.11.1). Apart from TAG analysis in the TRL fraction, all samples from each subject were analysed in the same batch. Accuracy and precision were maintained using quality-control sera (Roche Diagnostics Ltd.). Phenotypes of apolipoprotein E were determined by isoelectric focusing using Western blot techniques (section 3.11.3).

6.2.9 TRL fractionation
The TRL fraction (Sf > 20) was separated by preparative ultracentrifugation (section 3.11.4) in an Optima TLX ultracentrifuge (Beckman Instruments Ltd., High Wy-
combe, Bucks, UK) in a fixed-angle rotor (Beckman, TLA 100·4). The TRL fraction was separated by slicing the tube.

6.2.10 Determination of TRL apo B48 and apo B100 and serum RLP-cholesterol concentrations

TRL apo B48 and B100 concentrations were used as specific markers for the concentration of TRL particles of intestinal and hepatic origin, respectively. TRL apo B48 and B100 concentrations were quantified using analytical sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (section 3.11.5). RLP-cholesterol was determined in serum samples obtained in the basal state and 4 h postprandially using a novel immunoseparation kit technique (Jimro-II) made available from Japanese Immunoresearch Laboratories Company (section 3.11.6).

6.2.11 Calculations and statistics

Plasma or serum concentrations measured in the fasted state and summary measures of postprandial responses (AUCs) were compared using one-way ANOVA for repeated measures. When significant differences were identified, Tukey post hoc comparisons were performed. For RLP-cholesterol concentrations, intervention and time effects were determined using two-way ANOVA for repeated measures. For the calculation of mol apo E per apo B100-containing TRL particle and mol TAG per apo B100-containing TRL particle in the fasted state, fasting TRL concentrations of TAG, apo E and apo B100 were converted to mol/L units. The molecular weights of apo B100 and apo E were taken as 512 kD and 34 kD, respectively. For both calculations, the presence of a small number of apo B48-containing TRL particles in the fasted state was disregarded as these particles represent a very small proportion of the TRL pool and are relatively TAG-poor (Sf < 400) compared to “normal” chylomicrons (Karpe and Hamsten 1994). The mol TAG per apo B100-containing TRL particle in the fasted state was used as a measure of VLDL particle size. The proportion of TRL particles which was of endogenous origin (apo B100-containing) was calculated from the concentrations (mg/L) of apo B100 and apo B48 in the TRL fraction using the formula: [apo B100] / [apo B100] + [apo B48] / 0.48 (Karpe 1999). LDL cholesterol concentration in the fasted state was estimated using the Friedewald formula (Friedewald et al. 1972). Data for mol apo E per apo B100-
containing TRL particle and mol TAG per apo B100-containing TRL particle were not normally distributed and, therefore, they were transformed to their natural logarithm before statistical testing. Rates of whole body carbohydrate and fat utilisation and energy expenditure were calculated using indirect calorimetry, as described in section 3.3. A 5 % level of significance was adopted throughout. Statistical procedures were performed using Statistica for Windows, version 5.0 (Tulsa, OK, USA).

6.3 Results
6.3.1 Experimental diets and test meal
Both experimental diets were well tolerated, aside from a common complaint by the subjects of feeling excessively full during the high-carbohydrate diet. Compliance, assessed by food inventories and detailed discussions with subjects, was high. The test meal was also well tolerated by all subjects without any sign of nausea or other gastrointestinal discomfort. It was consumed over a median of 13 (range 11 – 15) minutes.

6.3.2 Cardiorespiratory and metabolic responses during exercise sessions
During the Hi-CHO-Ex intervention, subjects walked at 1.5 ± 0.1 m/s (mean ± SD) up a 3 ± 1 % gradient, with an average VO₂ of 17.7 ± 1.1 mL/kg/min, which represented 61 ± 3 % of estimated VO₂max. Heart rate averaged 128 ± 8 beat/min and ratings of perceived exertion 12 ± 2 (on a scale from 6 to 20), corresponding to “fairly hard”. Gross energy expenditure per session was 1.46 ± 0.10 MJ, 65 ± 10 % from carbohydrate and 35 ± 10 % from fat. On average, 56 ± 11 g of carbohydrate and 13 ± 3 g of fat were oxidized daily during treadmill walking.

6.3.3 Plasma, TRL and serum concentrations in the fasted state
Plasma TAG and TRL-TAG concentrations were significantly higher after the High-CHO intervention than after the Low-CHO intervention, as were TRL-apo B48, apo B100 and apo E concentrations (Table 6.5). Because concentrations of both TRL-TAG and TRL apo B100 were similarly affected by the High-CHO intervention, the number of TAG molecules per apo B100-containing TRL particle did not differ between the Hi-CHO and the Low-CHO intervention (Table 6.5). Thus, the High-CHO
intervention did not seem to have affected the size of VLDL particles. The VLDL particles constituted a very large proportion of the total pool of TRL after both the Low-CHO (98 %) and the High-CHO (97 %) interventions. The High-CHO intervention increased the apo E content of VLDL particles (Table 6.5) as well as the serum RLP-cholesterol concentration (Table 6.8). Although total cholesterol concentration was not significantly affected by the High-CHO intervention, HDL-cholesterol was reduced (Table 6.5).

The addition of exercise to the High-CHO diet significantly attenuated the diet-induced increases in plasma TAG, TRL-TAG and TRL concentrations of apo B48, apo B100 and apo E (Table 6.5). The addition of exercise also seemed to reduce fasting serum RLP-cholesterol concentration but this did not reach statistical significance \( (P = 0.09) \). Exercise had no statistically significant effect on plasma HDL-cholesterol concentration when compared with the Hi-CHO intervention (Table 6.5).

Overall there were no significant differences between the High-CHO-Ex and the Low-CHO interventions in any of the above parameters except HDL-cholesterol, which was significantly higher after the Low-CHO intervention.

The fasting plasma NEFA concentration was not different among interventions but 3-hydroxybutyrate concentration was somewhat lower after the High-CHO than after the Low-CHO intervention \( (P < 0.07) \) (Table 6.6). Plasma glucose was significantly lower after the High-CHO than after the Low-CHO intervention. Serum insulin was lower after the High-CHO-Ex than after the High-CHO intervention (Table 6.6).
<table>
<thead>
<tr>
<th></th>
<th>Low-CHO</th>
<th>High-CHO</th>
<th>High-CHO-Ex</th>
<th>P value&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAG, mmol/L</td>
<td>0.96 ± 0.12</td>
<td>1.58 ± 0.19&lt;sup&gt;$&lt;/sup&gt;</td>
<td>1.21 ± 0.10&lt;sup&gt;+&lt;/sup&gt;</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>TRL-TAG, mmol/L</td>
<td>0.37 ± 0.08</td>
<td>0.85 ± 0.14&lt;sup&gt;$&lt;/sup&gt;</td>
<td>0.55 ± 0.09&lt;sup&gt;+&lt;/sup&gt;</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>TRL-apo B48, mg/L</td>
<td>0.08 ± 0.05</td>
<td>0.44 ± 0.12&lt;sup&gt;$&lt;/sup&gt;</td>
<td>0.23 ± 0.09&lt;sup&gt;+&lt;/sup&gt;</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>TRL-apo B100, mg/L</td>
<td>9.36 ± 2.27</td>
<td>27.84 ± 5.31&lt;sup&gt;$&lt;/sup&gt;</td>
<td>17.63 ± 3.49&lt;sup&gt;+&lt;/sup&gt;</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>TRL-apo E, mg/L</td>
<td>3.97 ± 1.33</td>
<td>21.59 ± 6.12&lt;sup&gt;$&lt;/sup&gt;</td>
<td>10.30 ± 3.06&lt;sup&gt;+&lt;/sup&gt;</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>mol apo E per apo B100-containing TRL particle</td>
<td>5.43 ± 0.92</td>
<td>12.74 ± 4.11&lt;sup&gt;T&lt;/sup&gt;</td>
<td>10.47 ± 3.16&lt;sup&gt;T&lt;/sup&gt;</td>
<td>0.02</td>
</tr>
<tr>
<td>mol TAG per apo B100-containing TRL particle</td>
<td>25,369 ± 5,315</td>
<td>16,676 ± 1,815</td>
<td>22,510 ± 8,991</td>
<td>0.42</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.37 ± 0.22</td>
<td>6.37 ± 0.23</td>
<td>6.23 ± 0.18</td>
<td>0.44</td>
</tr>
<tr>
<td>LDL-cholesterol, mmol/L</td>
<td>4.50 ± 0.09</td>
<td>4.37 ± 0.17</td>
<td>4.36 ± 0.15</td>
<td>0.34</td>
</tr>
<tr>
<td>HDL-cholesterol, mmol/L</td>
<td>1.39 ± 0.09</td>
<td>1.25 ± 0.08&lt;sup&gt;$&lt;/sup&gt;</td>
<td>1.29 ± 0.08&lt;sup&gt;$&lt;/sup&gt;</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Table 6.5  Plasma and TRL fraction concentrations of lipids and apolipoproteins measured in the fasted state after the low-carbohydrate diet (Low-CHO), after the high-carbohydrate diet (High-CHO), and after the high-carbohydrate diet with the addition of 60 minutes daily moderate intensity exercise (High-CHO-Ex). n = 8 women, mean ± SEM.

<sup>1</sup> Overall significance of differences among the three interventions by one-way ANOVA for repeated measures.

<sup>$</sup> P < 0.01 vs Low-CHO, <sup>+</sup> P < 0.05 vs Low-CHO by Tukey post hoc test.

<sup>†</sup> P < 0.01 vs High-CHO, <sup>T</sup> P < 0.05 vs High-CHO by Tukey post hoc test.
<table>
<thead>
<tr>
<th></th>
<th>Low-CHO</th>
<th>High-CHO</th>
<th>High-CHO-Ex</th>
<th>P value $^{\dagger}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-esterified fatty acids, mmol/L</td>
<td>0.51 ± 0.05</td>
<td>0.49 ± 0.04</td>
<td>0.57 ± 0.04</td>
<td>0.18</td>
</tr>
<tr>
<td>3-Hydroxybutyrate, mmol/L</td>
<td>0.10 ± 0.02</td>
<td>0.07 ± 0.01</td>
<td>0.10 ± 0.01</td>
<td>0.06</td>
</tr>
<tr>
<td>Insulin, μU/mL</td>
<td>10.8 ± 1.3</td>
<td>12.3 ± 1.9</td>
<td>9.8 ± 1.1 $^{\dagger}$</td>
<td>0.04</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.25 ± 0.18</td>
<td>5.04 ± 0.14 $^{\dagger}$</td>
<td>5.06 ± 0.15</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Table 6.6 Plasma non-esterified fatty acids and glucose and serum 3-hydroxybutyrate and insulin concentrations measured in the fasted state after three days on a low-carbohydrate diet (Low-CHO), after three days on a high-carbohydrate diet (High-CHO), and after three days on the same high-carbohydrate diet with the addition of 60 minutes daily moderate intensity exercise (High-CHO-Ex). $n = 8$ women, mean ± SEM.

$^{\dagger}$ Overall significance of differences among the three interventions by one-way ANOVA for repeated measures.

$^{\dagger}$ $P < 0.05$ vs Low-CHO by Tukey post hoc test.

$^{\dagger}$ $P < 0.05$ vs Hi-CHO by Tukey post hoc test.
<table>
<thead>
<tr>
<th></th>
<th>Low-CHO</th>
<th>High-CHO</th>
<th>High-CHO-Ex</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma TAG, mmol/L × h</td>
<td>10.12 ± 1.15</td>
<td>13.74 ± 1.57§</td>
<td>10.54 ± 1.08†</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>TRL-TAG, mmol/L × h</td>
<td>8.68 ± 1.31</td>
<td>11.49 ± 1.26§</td>
<td>8.86 ± 1.09‡</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>TRL-apo B48, mg/L × h</td>
<td>2.88 ± 1.11</td>
<td>5.39 ± 1.41§</td>
<td>2.97 ± 0.66†</td>
<td>0.02</td>
</tr>
<tr>
<td>TRL-apo B100, mg/L × h</td>
<td>127 ± 29</td>
<td>216 ± 47§</td>
<td>143 ± 28†</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>TRL-apo E, mg/L × h</td>
<td>120 ± 36</td>
<td>246 ± 69§</td>
<td>128 ± 32‡</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Non-esterified fatty acids, mmol/L × h</td>
<td>3.15 ± 0.24</td>
<td>2.66 ± 0.16</td>
<td>2.95 ± 0.25</td>
<td>0.06</td>
</tr>
<tr>
<td>3-Hydroxybutyrate, mmol/L × h</td>
<td>0.89 ± 0.12</td>
<td>0.57 ± 0.08§</td>
<td>0.72 ± 0.10</td>
<td>0.03</td>
</tr>
<tr>
<td>Insulin, µIU/mL × h</td>
<td>194 ± 31</td>
<td>202 ± 32</td>
<td>159 ± 22†</td>
<td>0.01</td>
</tr>
<tr>
<td>Glucose, mmol/L × h</td>
<td>33.3 ± 1.3</td>
<td>33.7 ± 1.07</td>
<td>33.08 ± 1.27</td>
<td>0.42</td>
</tr>
</tbody>
</table>

Table 6.7 Six-hour areas under the plasma, TRL fraction or serum concentration vs time curves after three days on a low-carbohydrate diet (Low-CHO), after three days on a high-carbohydrate diet (High-CHO), and after three days on the same high-carbohydrate diet with the addition of 60 minutes daily moderate intensity exercise (High-CHO-Ex). n = 8 women, mean ± SEM.

1 Overall significance of differences among the three interventions by one-way ANOVA for repeated measures.

§ P < 0.01 vs Low-CHO, ‡ P < 0.05 vs Low-CHO by Tukey post hoc test.

† P < 0.01 vs Hi-CHO, † P < 0.05 vs Hi-CHO by Tukey post hoc test.
<table>
<thead>
<tr>
<th>RLP-cholesterol</th>
<th>Low-CHO</th>
<th>High-CHO</th>
<th>High-CHO-Ex</th>
<th>$P$ value$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h, mmol/L</td>
<td>0.26 ± 0.02</td>
<td>0.41 ± 0.07$^\dagger$</td>
<td>0.29 ± 0.02</td>
<td>0.04</td>
</tr>
<tr>
<td>4 h, mmol/L</td>
<td>0.38 ± 0.04</td>
<td>0.55 ± 0.06$^\S$</td>
<td>0.41 ± 0.04$^\Dagger$</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Table 6.8 Remnant-like lipoprotein particle (RLP) cholesterol concentration in the fasted state (0 h) and at 4 h after the consumption of the test meal after three days on a low-carbohydrate diet (Low-CHO), after three days on a high-carbohydrate diet (High-CHO), and after three days on the same high-carbohydrate diet with the addition of 60 minutes daily moderate intensity exercise (High-CHO-Ex). $n = 8$ women, mean ± SEM.

$^1$ Overall significance of differences among the three interventions by one-way ANOVA for repeated measures.

$^\S P < 0.01$ vs Low-CHO, $^\dagger P < 0.05$ vs Low-CHO by Tukey post hoc test.

$^\Dagger P < 0.05$ vs Hi-CHO by Tukey post hoc test.
6.3.4 Postprandial responses

Plasma TAG and TRL-TAG concentrations after the high-fat, mixed meal are shown in Figure 6.1. The AUCs for postprandial plasma TAG and TRL-TAG were 36 % and 32 % (respectively) greater after the High-CHO than after the Low-CHO intervention (Table 6.7). However, the addition of daily exercise to the High-CHO intervention significantly reduced both AUCs, almost to the level observed after the Low-CHO intervention (Table 6.7).

Postprandial concentrations of apo B48, apo B100 and apo E in the TRL fraction are shown in Figure 6.2. As in the fasted state, the apo B100-containing particles constituted a very large proportion of the TRL particles (for example, at 4 h postprandially, 94 - 95 % after both diet-only interventions). The AUCs for all measured apolipoproteins were significantly higher after the High-CHO than after the Low-CHO intervention (Table 6.7). After the High-CHO-Ex intervention the AUCs for all three apolipoproteins were significantly lower than after the High-CHO intervention (Figure 6.2, Table 6.7).

RLP-cholesterol concentration increased postprandially (main effect of time: $P < 0.01$) (Table 6.8). The Hi-CHO intervention led to 50 % higher postprandial concentrations than the Low-CHO intervention, but exercise prevented this increase. The RLP-cholesterol concentrations were highly related to the plasma TAG concentrations ($r = 0.88$, $P < 0.01$).

The insulin and glucose, NEFA and 3-hydroxybutyrate responses are shown in Figure 6.3 and 6.4, respectively. Their summary measures (AUCs) are given in Table 6.7.
Figure 6.1  Plasma TAG and TRL-TAG concentrations in the fasting state (0 hour) and for six hours following consumption of a high-fat mixed meal after three days on a low-carbohydrate diet (Low-CHO), after three days on a high-carbohydrate diet (High-CHO) and after three days on the same high-carbohydrate diet with the addition of 60 minutes daily moderate intensity exercise (High-CHO-Ex). \( n = 8 \) women. Mean ± SEM.
Figure 6.2  TRL-apo B48 and B100 concentrations in the fasted state (0 hour) and for six hours following consumption of a high-fat mixed meal after three days on a low-carbohydrate diet (Low-CHO), after three days on a high-carbohydrate diet (High-CHO) and after three days on the same high-carbohydrate diet with the addition of 60 minutes daily moderate intensity exercise (High-CHO-Ex). $n = 8$ women. Mean ± SEM.
Figure 6.3  TRL-apo E concentrations in the fasted state (0 hour) and for six hours following consumption of a high-fat mixed meal after three days on a low-carbohydrate diet (Low-CHO), after three days on a high-carbohydrate diet (High-CHO) and after three days on the same high-carbohydrate diet with the addition of 60 minutes daily moderate intensity exercise (High-CHO-Ex). n = 8 women. Mean ± SEM.
Figure 6.4  Serum insulin and plasma glucose concentrations in the fasted state (0 hour) and for six hours following consumption of a high-fat mixed meal after three days on a low-carbohydrate diet (Low-CHO), after three days on a high-carbohydrate diet (High-CHO) and after three days on the same high-carbohydrate diet with the addition of 60 minutes daily moderate intensity exercise (High-CHO-Ex). n = 8 women. Mean ± SEM.
Figure 6.5  Plasma non-esterified fatty acids (NEFA) and serum 3-hydroxybutyrate concentrations in the fasted state (0 hour) and for six hours following consumption of a high-fat mixed meal after three days on a low-carbohydrate diet (Low-CHO), after three days on a high-carbohydrate diet (High-CHO) and after three days on the same high-carbohydrate diet with the addition of 60 minutes daily moderate intensity exercise (High-CHO-Ex). \( n = 8 \) women. Mean ± SEM.
6.3.5 Indirect calorimetry

Fasting and postprandial respiratory exchange ratio (RER) values are presented in Figure 6.6. Neither the High-CHO nor the High-CHO-Ex intervention significantly affected fasting or postprandial energy expenditure and substrate oxidation. Details are given below.

In the fasted state, RER values were similar after the three different interventions: 0.82 ± 0.03 (Low-CHO), 0.83 ± 0.03 (High-CHO) and 0.82 ± 0.02 (High-CHO-Ex). Calculated rates of fat utilisation were 0.06 ± 0.01 g/min (Low-CHO), 0.05 ± 0.02 g/min (High-CHO) and 0.05 ± 0.02 g/min (High-CHO-Ex) and were not significantly different among interventions. The same was the case for rates of carbohydrate utilisation, which were 0.11 ± 0.03 g/min (Low-CHO), 0.16 ± 0.05 g/min (High-CHO) and 0.14 ± 0.06 g/min (High-CHO-Ex). Therefore, calculated resting metabolic rate did not differ significantly among interventions: 4.04 ± 0.57 kJ/min (Low-CHO), 4.49 ± 0.64 kJ/min (High-CHO) and 4.22 ± 0.61 kJ/min (High-CHO-Ex).

In the postprandial state, calculated energy expenditure over the 6-hour observation period was not significantly different among interventions: 1.69 ± 0.04 MJ (Low-CHO), 1.61 ± 0.04 MJ (High-CHO) and 1.75 ± 0.06 MJ (High-CHO-Ex). The amount of fat utilised during the 6-h postprandial period was 32 ± 3 g (Low-CHO), 30 ± 4 g (High-CHO) and 33 ± 2 g (High-CHO-Ex) (NS). Corresponding values for the amount of carbohydrate utilised postprandially were 27 ± 5 g, 26 ± 7 g and 21 ± 4 g (NS).
Figure 6.6  Respiratory exchange ratio (RER) values in the fasted state (0 hour) and for six hours following consumption of a high-fat mixed meal after three days on a low-carbohydrate diet (Low-CHO), after three days on a high-carbohydrate diet (High-CHO) and after three days on the same high-carbohydrate diet with the addition of 60 minutes daily moderate intensity exercise (High-CHO-Ex). \( n = 8 \) women. Mean ± SEM.
6.4 Discussion
The findings of the present study confirmed the potential of daily moderate intensity exercise to prevent the augmentation of postprandial lipaemia attributable to a low-fat, high-carbohydrate diet. More importantly, it was demonstrated that this type of exercise can exert its beneficial effect on TAG metabolism in the population of postmenopausal women which is at a higher risk for CHD (Hokanson and Austin 1996; Kannel et al. 1976) than the population of young men studied in the previous study (chapter 5). In the present study, direct quantifications of TRL-apo B48, TRL-apo B100 and serum remnant lipoproteins were performed after high-carbohydrate diet and exercise interventions. The data suggest that daily moderate intensity exercise can reverse the increases in fasting and postprandial concentrations of TRL apo B48 and apo B100 and in serum remnant lipoproteins induced by a short-term high-carbohydrate diet.

In the fasted state, the high-carbohydrate diet resulted in higher plasma TAG and TRL-TAG concentrations compared to the low-carbohydrate diet. It has been suggested that both elevated TAG synthesis and inadequate or reduced TAG clearance could contribute to carbohydrate-induced hypertriacylglycerolaemia (Parks and Hellerstein 2000). In the present study increases in fasting TRL-TAG concentrations after the high-carbohydrate diet were accompanied by increases in apo B100 concentrations, indicating the presence of more, rather than bigger, VLDL particles (Table 6.5). An interesting observation was the increased presence of apo B48-containing TRL particles in the 12-hour fasted state after the high-carbohydrate diet. This was surprising, taking into consideration the very small amount of fat the subjects had consumed the day before (~35 g). These particles may constitute a certain subpopulation of chylomicron remnants with a very slow turnover (Karpe et al. 1997a; Welty et al. 1999). A prolongation of their residence time in the circulation could also have resulted from increased competition with VLDL particles that were elevated 3-fold in the fasted state after the high-carbohydrate diet.

In the postprandial state, the high-carbohydrate diet resulted in plasma TAG and TRL-TAG responses that were more than one third greater than after the low-carbohydrate diet. Additionally, after the high-carbohydrate diet, the responses of
apo B48 and apo B100 in the TRL fraction were 87% and 70% higher, respectively. The removal of TAG from chylomicrons and VLDL is achieved by the same enzymatic pathway (Brunzell et al. 1973; Björkegren et al. 1996). Thus, increases in the concentration of TRL particles in the fasted state, especially of VLDL particles, which are much more numerous than apo B48-containing particles, could increase competition for LPL dramatically, when newly synthesised chylomicrons appear postprandially. This will impede degradation of both species of TRL particles and lead to their accumulation. To exacerbate the situation further, (heparin releasable) LPL activity has been reported to be significantly reduced by high-carbohydrate diets (Campos et al. 1995). Thus, after the high-carbohydrate diet more TRL particles could have been competing postprandially for a “less active” lipolytic pathway.

The high-carbohydrate diet led to more apo E-enriched VLDL particles in the fasted state and an exaggerated and protracted apo E postprandial response compared to the low-carbohydrate diet. In the postprandial state, a transient increase in apo E concentration in the TRL fraction is expected because of the redistribution of apo E from HDL to the TRL fraction (Blum 1982). Apo E allows for the uptake and recognition of TRL remnants by a receptor-dependent process in the liver (Beisiegel 1995) and as a result, apo E levels decrease again. According to this reasoning, the high postprandial apo E concentrations after the high-carbohydrate diet, especially at the late time points, probably reflect sustained levels of TRL remnants that were not taken up efficiently by the liver and, therefore, had increased residence time in the circulation. This interpretation is supported by the 40% higher RLP-cholesterol concentration at 4 hours postprandially after the high- than the low-carbohydrate diet, suggesting higher levels of cholesteryl-ester-enriched remnant lipoproteins.

The observed carbohydrate-induced alterations to TAG and TRL metabolism are potentially atherogenic. First, high fasting (Hokanson and Austin 1996) and postprandial (Patsch et al. 1992) TAG concentrations have been recognised as an independent risk marker of CHD. Therefore, the observed carbohydrate-induced elevations may indicate a potentially atherogenic impairment in lipid metabolism. Second, the observed, diet-induced increases in the number of apo B100-containing TRL particles (VLDL), especially in the postprandial state, could also confer a high
atherogenic risk. VLDL particles (as well as chylomicrons, although much fewer in number) become particularly enriched in cholesterol postprandially and this may increase their atherogenic potential (Björkgren et al. 1998). Indeed, in a previous study comparing the pattern of TRL particles in the postprandial state in subjects with and without manifest coronary atherosclerosis, the postprandial accumulation of large VLDL was the most pronounced discriminator between the groups (Karpe et al. 1999). Furthermore, cholesterol and apo E-enriched VLDL particles have been isolated from human atherosclerotic plaque (Rapp et al. 1994). Another potentially adverse effect of the high-carbohydrate diet in relation to the risk of CHD is the elevation in RLP-cholesterol concentration. RLP-cholesterol has been found to be increased in normolipidaemic men with CHD (Devaraj et al. 1998) and may be useful in the assessment of CHD risk. In the present study the high-carbohydrate diet increased the RLP-cholesterol levels by almost 60% in the fasted state and 40% postprandially compared with the low-carbohydrate diet. These increases probably indicate higher levels of cholesteryl-ester-enriched TRL remnants after the high-carbohydrate diet that are potentially atherogenic.

If all the above carbohydrate-induced alterations to lipoprotein metabolism confer a high atherogenic risk, then the addition of exercise largely offsets this. Exercise prevented the augmentation of postprandial lipaemia and the accumulation of TRL particles both in the fasted and postprandial states. Exercise could oppose the overproduction of VLDL-TAG (Sidossis and Mittenddorfer 1999) and/or the suppressive effects on LPL activity (Campos et al. 1995) of a diet high in carbohydrate. Both these potential mechanisms have been discussed in chapter 5. These exercise-induced changes could have decreased the competition between VLDL and chylomicrons and improved the capacity for hydrolysis of their TAG content. The addition of exercise to the high-carbohydrate diet did not reduce the enrichment of VLDL with apo E in the fasted state but it did prevent the exaggerated and protracted postprandial apo E response observed with the high-carbohydrate diet. The latter effect possibly reflects an effective clearance of TRL remnants, which is supported by the lower postprandial RLP-cholesterol levels.
Whether low-fat, high-carbohydrate diets provide a net benefit with respect to CHD risk is a key and unresolved public health issue (Parks and Hellerstein 2000). In the present study, fasting hypertriacylglycerolaemia and exaggerated postprandial lipaemia were observed after exchanging dietary fat with carbohydrate. These effects were associated with increases in the numbers of circulating TRL particles and their potentially atherogenic remnants but were abolished by daily, moderate exercise. These findings strongly suggest that regular exercise during the consumption of a low-fat, high-carbohydrate diet may optimise CHD risk reduction.
Coronary heart disease is the leading cause of death in the affluent Western societies (Murray and Lopez 1997). There is a general agreement that diets with a high proportion of saturated fatty acids raise LDL-cholesterol levels and consequently, increase the risk of CHD. However, what macronutrient should replace saturated fatty acids in the diet remains controversial. Whilst some scientists stress that the type of fat, rather than the total amount, is of prime importance in CHD risk (Katan 1997), some others advocate a reduction in total fat with replacement from carbohydrate (Connor and Connor 1997). Most public health recommendations have focused on low-fat, high-carbohydrate diets because populations with low intakes of saturated and total fat tend to be at low risk and, also, because these diets effectively decrease total and LDL-cholesterol levels (Grundy et al. 1982). However, low-fat, high-carbohydrate diets also raise fasting TAG and reduce HDL-cholesterol concentrations (Mensink and Katan 1992). As high TAG and low HDL-cholesterol concentrations independently increase the risk (Hokanson and Austin 1996), the value of replacing fat with carbohydrates has been questioned (Roche 2000). One theoretical argument in favour of the anti-atherogenic utility of high-carbohydrate diets has been that postprandial lipaemia will be lower during these diets as the amount of ingested dietary fat is reduced (Connor and Connor 1997).

Postprandial lipaemia is transient hypertriacylglycerolaemia, which affects the physical characteristics and particle composition of all major plasma lipoproteins in a way that it may contribute to the initiation and progression of atherosclerosis. In fact, the magnitude of postprandial lipaemia may be critical as it seems to determine, at least partly, fasting TAG (Griffin 1997) and HDL-cholesterol (Lechleitner et al. 1990) levels, as well as the size of population of small dense LDL particles (Karpe et al. 1993b) that are potentially atherogenic (Griffin 1999). In addition, TRL particles may directly enhance atherosclerosis, taking into consideration the compositional characteristics they attain postprandially (Björkegren et al. 1997; Björkegren et al. 1998) and that TRL particles have been isolated from human atherosclerotic plaque (Rapp et al. 1994).
In the preceding three chapters of this thesis, a series of investigations have been presented examining the effect of low-fat, high-carbohydrate diets on postprandial lipaemia. In an attempt to isolate the effects of the interventions from the acute effects of macronutrients in the test meal on postprandial lipaemia, an identical test meal was employed after each intervention. As opposed to the theoretical argument in favour of the anti-atherogenic utility of high-carbohydrate diets (Connor and Connor 1997), it was found that high-carbohydrate diets were associated with fasting hypertriacylglycerolaemia and augmented postprandial lipaemia compared to isoenergetic diets lower in carbohydrate and higher in fat. Specifically, a 68% carbohydrate diet increased postprandial lipaemia (TAG-AUC) by 90% compared with a 15% carbohydrate diet in healthy men (chapter 4). Almost the same diet (70% carbohydrate) increased postprandial lipaemia by 35% compared with a 46% carbohydrate diet in men (chapter 5) or compared with a 35% carbohydrate diet in healthy postmenopausal women (chapter 6). In the study described in chapter 6, the qualitative changes to postprandial TAG metabolism elicited by high-carbohydrate diets were also investigated. It was observed that exchanging dietary fat with carbohydrate was associated with increases in the numbers of circulating apo B48- and apo B100-containing TRL particles and their remnants in both the fasted and postprandial states. Specifically, the 70% carbohydrate diet was associated with increases in the postprandial responses (AUC) of TRL apo B48- and apoB100-containing lipoproteins by 87% and 70%, respectively.

The potential mechanisms by which high-carbohydrate diets cause hypertriacylglycerolaemia and postprandial accumulation of TRL particles have been discussed in section 2.8.4 and chapters 4, 5 and 6. An overview of these mechanisms is provided here. Carbohydrate-induced fasting hypertriacylglycerolaemia appears to result from overproduction of both VLDL-TAG and VLDL particles (Parks and Hellerstein 2000); clearance of VLDL could also be impaired (Parks et al. 1999). Accumulation of VLDL particles in the fasted state probably increases the competition with chylomicrons for hydrolysis by LPL postprandially (Brunzell et al. 1973; Karpe and Hultin 1995; Björkergren et al. 1996), leading to postprandial accumulation of both TRL species and their remnants. The latter was clearly indicated in chapter 6 by the
exaggerated and delayed postprandial response of TRL apo E and high levels of RLP-cholesterol after the high-carbohydrate diet.

Hepatic de novo lipogenesis per se is not likely to be a primary factor of carbohydrate-induced hypertriacylglycerolaemia. However, as discussed in section 2.8.4.1 and chapters 4 and 5, de novo lipogenesis could provide a signal within the hepatocyte to shift more plasma-derived fatty acids from oxidation toward esterification and TAG synthesis. According to studies in rats, this effect on hepatic fat oxidation seems to be linked to an inhibition of carnitine acyl-transferase I, located on the outer side of the mitochondrial inner membrane, by malonyl CoA, the first committed intermediate of lipogenesis (McGarry and Foster 1980). However, a variation of malonyl CoA concentration is not always responsible for the decrease in hepatic fatty acid oxidation when lipogenic rate increases (Decaux et al. 1988). It may involve, in addition, a decrease in carnitine acyltransferase I activity and an increase in the sensitivity of this enzyme to malonyl CoA. Whatever the mechanism is, hepatic fat oxidation would be decreased and more fatty acids would be shunted toward VLDL production. The present thesis provided some evidence, albeit indirect, for a reduced hepatic fatty acid \( \beta \)-oxidation after high-carbohydrate diets by demonstrating lower serum 3-hydroxybutyrate concentrations in the fasted (chapter 4) and postprandial states (chapters 4, 5 and 6) after the high-carbohydrate diet than after diets lower in carbohydrate.

Certainly, a defect in TRL-TAG hydrolysis can also constitute a mechanism for the observed carbohydrate-induced hypertriacylglycerolaemia and kinetic studies have provided evidence for this (Parks et al. 1999; Chen et al. 1995). However, the mechanism for this defect is less clear as is the exact contribution of LPL, taking into consideration that a reduction in VLDL-TAG fractional catabolic rate after a high-carbohydrate diet has been found to be accompanied by a significant increase in postheparin plasma LPL activity (Chen et al. 1995). Theoretically, the degree of activity of LPL in postheparin plasma indicates the upper limit for the rate of hydrolysis of TRL-TAG in plasma. Previous studies (Karpe 1992; Karpe et al. 1993a), however, provided little evidence that this measure is metabolically important. No significant relations were found between postheparin plasma LPL activity and AUC-
TAG measurements in the $S_f > 400$, $S_f 60-400$ and $S_f 20-60$. Furthermore, only weak negative correlation coefficients were obtained between postheparin plasma LPL activity and the AUCs for $S_f 60-400$ apo B48 and apo B100. Concerning the $S_f 20-60$ fraction, no significant relations with AUCs for apo B48 and apo B100 and postheparin plasma LPL activity were noted. There is currently no way to assess LPL activity in vivo without the use of heparin, which, as discussed above, may not provide physiologically relevant results. The mechanisms responsible for reduced TRL-TAG or/and VLDL-TAG overproduction on low-fat, high-carbohydrate diets await further study.

Taken together with clinical data, the changes in lipoprotein metabolism induced by euenergetic high-carbohydrate diets appear to support concerns about the atherogenic potential of these diets. In the studies described in this thesis, it was found that the high-carbohydrate diets were associated with fasting hypertriacylglycerolaemia and exaggerated postprandial lипаemia. These effects were accompanied by increases in the number of circulating TRL particles of both exogenous and endogenous origin. Fasting TAG concentration is an independent risk factor for CHD (Hokanson and Austin 1996) while there is also accumulated evidence that elevated postprandial levels of plasma TAG and TRL particles are closely related to the risk of CHD and early atherosclerosis (Karpe 1999). As discussed in chapter 6, the carbohydrate-induced increases in the number of apo B100-containing TRL particles (VLDL), especially in the postprandial state, could confer a high atherogenic risk. VLDL particles (as well as chylomicrons, although much fewer in number) become particularly enriched in cholesterol postprandially and this may increase their atherogenic potential (Björkegren et al. 1998). Another potentially adverse effect of high-carbohydrate diets is the accumulation of remnant lipoproteins as was indicated by the elevation in RLP-cholesterol concentration (chapter 6). There is considerable evidence linking TRL remnant particles with the risk or progression of atherosclerosis (Hodis and Mack 1998; Hodis 1999). Collectively, these observations lead to the concern that, at least in some individuals, the beneficial LDL-cholesterol lowering effect of high-carbohydrate diets may be offset by adverse changes in other circulating lipoprotein fractions (Baum and Brown 2000).
Probably, the most striking finding of the research in this thesis was the potential of exercise to abolish the effects of high-carbohydrate diets on TAG metabolism. It was clearly demonstrated that the TAG-lowering effect of exercise (Aldred et al. 1994; Tsetsonis et al. 1997; Gill and Hardman 2000) is sufficiently potent to offset the augmentation of postprandial lipaemia, which develops when subjects change to a low-fat, high-carbohydrate diet. Thirty minutes of daily moderate exercise was sufficient to negate the carbohydrate-induced augmentation of postprandial lipaemia, although not the increase in fasting TAG levels, in healthy young men (chapter 5). This study showed the importance of studying humans in the fed state and the dissociation that may exist between fasting and postprandial TAG concentrations. Furthermore, one hour of daily exercise entirely prevented the accumulation of TAG, TRL particles and their remnants in both the fasted and postprandial states in healthy postmenopausal women (chapter 6). Importantly, the exercise in both studies (chapters 5 and 6) was prescribed at a "relative exercise intensity" of 60 % \( \text{VO}_{2} \text{max} \), which is considered as moderate by most classifications. Thus, each individual exercised at a speed and gradient, which he/she could manage comfortably. As the exercise was prescribed according to individual ability and fitness, the least fit subjects performed less exercise in absolute terms than their fitter counterparts but even these subjects experienced substantial reductions in plasma TAG concentrations. Interestingly, the dietary change that was employed was more extreme in the proportion of dietary energy from carbohydrate (70 % carbohydrate) than that recommended to the population at large (e.g. > 55 % carbohydrate, National Cholesterol Education Program Step I diet). Nonetheless, one hour and even 30 minutes of moderate intensity exercise daily was sufficient to negate the carbohydrate-induced augmentation of postprandial lipaemia. A smaller amount of exercise is, therefore, likely to offset the effects of a more modest dietary change.

The potential mechanisms by which exercise prevented the effects of carbohydrate-rich diets on TAG metabolism have been discussed in chapters 5 and 6 and an overview is given here. One possible explanation may be that exercise opposed the reduction in the capacity for clearance of TAG that has been demonstrated after a high-carbohydrate diet (Parks et al. 1999). This exercise effect may have been mediated by increases in the activity of LPL located at the endothelial surface of capillaries, which can be measured with the use of heparin. Taking into consideration
the already discussed concerns on whether heparin-releasable LPL activity provides physiologically relevant results, both a single exercise session (Kantor et al. 1987) and exercise conditioning (Weintraub et al. 1989) increase postheparin plasma LPL activity. In addition, they both enhance the capacity for intravenous fat clearance (Sady et al. 1986; Thompson et al. 1988). These exercise-induced changes may have affected postprandial TAG metabolism, when exercise was combined with the high-carbohydrate diet. It should be noted, however, that in all four studies just referred to, the intensity of exercise was higher than in the studies described in this thesis. Specifically, exercise was either at 80 % maximum heart rate (> 60 % \( \dot{V}O_2 \max \)) (Kantor et al. 1987; Weintraub et al. 1989; Thompson et al. 1988), or intense and prolonged (marathon running) (Sady et al. 1986). To what extent one hour or even 30 minutes of daily exercise at 60 % \( \dot{V}O_2 \max \) affected TAG clearance capacity is unclear. It may be that it was enough to oppose a reduction due to the carbohydrate-rich diet.

Another possible explanation for the TAG-lowering effect of exercise in the fasted and postprandial state may be a suppressive effect on carbohydrate-induced VLDL overproduction. Studies in rats have shown that exercise decreases hepatic VLDL production (Simonelli and Eaton 1978; Mondon et al. 1984; Fukuda et al. 1991) even during long-term carbohydrate feeding (Zavaroni et al. 1981). The causative factors for the lowering effect of exercise on VLDL-TAG-production could be, at least partly, an alteration in the hepatic partitioning of long-chain fatty acids between esterification and oxidation (Fukuda et al. 1991). High-carbohydrate diets (Sidossis and Mittenddorfer 1999) and increased acute glucose availability (Sidossis et al. 1998) decrease hepatic fatty acid oxidation, possibly by direct inhibition of long-chain fatty acid entry into the mitochondria via carnitine acyl-transferase I (section 2.8.4.1). Hypothetically, exercise could reduce or oppose this effect by diverting a considerable amount of carbohydrate to muscles for use as an energy source and for glycogen replenishment in the post-exercise state (Hamilton et al. 1996). In this way, the amount of carbohydrate that needs to be disposed of by the liver would be reduced, possibly reducing malonyl CoA production. This, in turn, may relieve the inhibition on carnitine acyl-transferase I, thereby allowing the flow of long-chain fatty acids into the mitochondria for \( \beta \)-oxidation and decreasing the availability of fatty acids for TAG synthesis. There is some evidence from the re-
search in this thesis (chapters 5 and 6) for an exercise-induced increase in hepatic fatty acid $\beta$-oxidation. The 3-hydroxybutyrate response (AUC) to the test meal was higher when the high-carbohydrate diet was combined with exercise than after the high-carbohydrate diet alone, however, this increase did not reach statistical significance. Whether exercise attenuates VLDL-TAG secretion in humans on a high-carbohydrate diet and to what extent this is determined by an effect on the hepatic partitioning of long-chain fatty acids between esterification and oxidation requires investigation.

The present work demonstrated that daily moderate exercise can offset, in the short term, the potentially detrimental effects of high-carbohydrate diets on postprandial TAG and TRL metabolism. Certainly, intervention studies over a longer time-scale are needed to examine whether exercise exerts the same effect in the long term. Hypothetically, a normalisation of TRL metabolism by exercise during low-fat, high-carbohydrate diets could also prevent the effects of these diets in other lipoprotein species such as the LDL particles. Previous reports have shown that more than a third of men who manifested a LDL subclass phenotype A (large, buoyant LDL) while consuming a high-fat diet converted to phenotype B (small, dense LDL) with a reduction in fat to 20-24 % of energy and a concomitant increase in carbohydrate (Dreon et al. 1994; Krauss and Dreon 1995). A subsequent study by the same group (Dreon et al. 1999) demonstrated that an additional 32 % of men who had stable phenotype A during the low-fat, high-carbohydrate converted to phenotype B after consumption of a very-low-fat (10 % fat), high-carbohydrate diet. Several studies have shown that risk of CHD is significantly greater in individuals with a predominance of small, dense LDL particles (Austin et al. 1988; Griffin 1994; Gardner et al. 1996). Thus, it seems that low-fat, high-carbohydrate diets can increase the risk for CHD in individuals who are considered at low risk while consuming their usual diets. It was also noted (Dreon et al. 1999) that after the consumption of the high-carbohydrate diet increases in concentration of dense LDL-III and reductions in buoyant LDL-I particles were inversely correlated with increases in fasting TAG concentrations. Furthermore, these subjects whose LDL phenotype changed from A to B after the high-carbohydrate diet had two-fold higher concentrations of plasma fasting TAG and higher concentrations of all VLDL subclasses, particularly the
largest VLDL particles, compared with the stable A group. With regard to the metabolic basis for the shift in LDL subclass phenotype from A to B, these authors speculated that the carbohydrate-induced increases in TAG and TRL concentrations may have resulted in TAG enrichment of LDL particles by the action of CETP and their subsequent remodeling and conversion to small dense LDL by hepatic lipase, however, some genetic influence was probably also involved. The magnitude of postprandial lipaemia has been positively associated with the levels of small dense LDL in the fasted state (Karpe et al. 1993b). Although no postprandial investigations were conducted in the above mentioned studies, it may be that an exaggeration in postprandial TAG and TRL concentrations due to the high-carbohydrate (as can be predicted by high fasting levels) particularly triggered the formation of small dense LDL particles during this diet. The research in this thesis showed that exercise during a high-carbohydrate diet prevents increases in fasting and postprandial TAG and TRL concentrations and, therefore, exercise might provide one means for attenuating the potentially deleterious effects of this type of diet on LDL subclasses.

In the study presented in chapter 6, one hour of daily moderate intensity exercise did not prevent the reduction in HDL-cholesterol concentration associated with the high-carbohydrate diet, although it did decrease fasting and postprandial TAG and TRL concentrations. There are several explanations for this. First, it has been suggested that a high level of physical activity leads to higher plasma HDL-cholesterol concentration by increasing TAG metabolic capacity (Wood and Haskell 1979). It may be that the initial effect of exercise was to enhance postprandial TAG metabolism and that changes in HDL-cholesterol concentration would develop later. In this way, the duration of the intervention might have been too short for the exercise to exert an effect on HDL-cholesterol levels. Second, as discussed in section 2.7.2, there may be a dissociation of postprandial TAG metabolism and fasting HDL-cholesterol concentration at low or normal levels of postprandial lipaemia. Cohen and Grundy (1992) reported similar postprandial lipaemia in two groups of normo-lipidaemic men with distinctly different HDL-cholesterol levels (~0.8 mmol/L vs ~1.2 mmol/L). Thus, it may be that the similar postprandial TRL particle concentration and magnitude of lipaemia after the low-carbohydrate intervention and after the high-carbohydrate diet plus exercise intervention in the present work would not
necessarily confer similar HDL-cholesterol concentrations. Finally, it has recently been demonstrated that the decrease in HDL-cholesterol during an euenergetic low-fat, high-carbohydrate diet (National Cholesterol Education Program Step II diet) paralleled the reduction in apo AI (major apolipoprotein component of HDL particle) secretion rate (Vélez-Carrasco et al. 1999). It was, therefore, suggested that the requirement for HDL-mediated cholesterol removal may be less at lower intakes of dietary fat and cholesterol and so the carbohydrate-induced reduction in HDL-cholesterol levels should not be interpreted as a negative outcome with regard to CHD (Vélez-Carrasco et al. 1999). If this holds true, then the HDL-cholesterol-lowering effect of high-carbohydrate diets could indeed be independent of the effect of these diets on postprandial TAG metabolism. In contrast, a diet high in fat has been shown to increase apo AI secretion rate and HDL-cholesterol concentration compared with a low fat diet in transgenic mice expressing human apo AI (Hayek et al. 1993). It was suggested that the HDL-cholesterol-raising effect of high-fat diets represents a defensive response which along with higher secretion rates of apo AI may be a protective mechanism against the deleterious effects of elevated levels of apo B-containing particles on the arterial wall (Wolf 1996; Hayek et al. 1993).

Interventions of longer duration (> three weeks) than the three-day model used in the present work have provided some evidence that regular exercise may offset the reductions in HDL-cholesterol concentration attributable to high-carbohydrate diets. A recent meta-analysis (Yu-Poth et al. 1999) found that regular exercise attenuated the lowering effect of low-fat, high-carbohydrate diets (National Cholesterol Education Program Step I and Step II diets) on HDL-cholesterol concentration by inducing a 10-fold smaller decrease than the non-exercise interventions. It was speculated that the attenuating effect of exercise may be mediated by the weight loss which usually occurs with exercise regimens. This speculation is confirmed by a study in older glucose intolerant subjects (Hughes et al. 1994). This study demonstrated that a combination of a high-carbohydrate diet and moderate exercise for 12 weeks without significant changes in body composition and only small (~1 kg) changes in body weight was not effective in preventing the decrease in HDL-cholesterol that occurred with the high-carbohydrate diet. In contrast, the addition of weight loss (~10 kg) to the American Heart Association Step I diet (< 30 % fat) prevented the de-
crease in HDL-cholesterol seen with the same diet intervention alone in obese men (Dengel et al. 1995). Using a weight-reducing National Cholesterol Education Program Step I diet for one year in overweight men, Wood et al. (1991) found that, although the diet alone did not affect HDL-cholesterol concentration, the addition of aerobic exercise induced a significant elevation in HDL-cholesterol concentration which was accompanied by a greater weight loss (~9 kg) than the weight-reducing diet alone (~5 kg). However, a study by Nieman et al. (1990) showed that, despite a similar weight loss (~6 kg) after a five-week low-fat (30 % fat) diet and after the same diet combined with moderate exercise, the exercise prevented the decrease in HDL-cholesterol concentration induced by the low-fat diet alone. Clearly, more research is needed to clarify whether the exercise-induced attenuation in the reduction in HDL-cholesterol concentration due to low-fat, high-carbohydrate diets is dependent on changes in body weight (and composition) or not.

In the research of this thesis, postprandial investigations were conducted in subjects who had fasted for 12 hours overnight and consumed a single, standardised mixed meal containing large amounts of fat that ranged from 60 to 95 g. A similar model has been used in a number of postprandial studies as it provides some advantages. First, it defines the fasted state as a 12 hour overnight fast, minimising effects of previous meals on the “baseline” TAG concentration. Second, high fat doses exaggerate the metabolic sequelae of the postprandial response allowing better understanding of the biochemical basis of the postprandial TAG and TRL response. However, recently, some criticisms have been expressed that this approach for investigation of postprandial TAG metabolism may not provide results relevant to a free-living situation (Roche and Gibney 2000). Specifically, it has been reported (De Castro 1987) that the mean fat intake at each eating occasion throughout the day in free-living individuals is between 12 and 30 g, the fat dose being low in the early morning and higher in the early evening. These data may challenge the physiological relevance of some postprandial investigations because (i) the quantities of fat consumed as part of a habitual diet are much lower than those used in postprandial investigations, including these in the present work (ii) individuals consume dietary fat as part of three to six occasions rather than as one bolus, and (iii) most postprandial investigations begin in early morning, a stage in the circadian rhythm when fat in-
take tends to be low. Postprandial investigations using large fat loads are important in examining the mechanisms behind postprandial TAG metabolism. However, they may need to be complemented by investigations using more realistic models, before extrapolating any results to free-living situations. Different but complementary models could collectively provide a body of evidence which will progress knowledge on postprandial lipoprotein metabolism and lead to changes in public health policy and recommendations.

The findings of the present research regarding the effects of high-carbohydrate diets on TAG metabolism do not necessarily speak against current population recommendations for some substitution of energy from fat with carbohydrate, not least because the carbohydrate-rich diets employed were euenergetic, extreme and contained large amounts of sugars. High-carbohydrate foods exert a strong, positive effect on satiety (Blundell et al. 1994) and in free-living conditions may therefore aid weight reduction. Indeed, most of the subjects in the present thesis experienced some difficulty eating all the food prescribed for the isonertgetic, high-carbohydrate diet. Given a choice, they would have consumed less food, possibly resulting in energy deficit and speculatively, in the long term, in weight loss. In line with this reasoning, several experimental studies have demonstrated significant weight loss during the consumption of ad libitum low-fat, high-carbohydrate diets (Schaefer et al. 1995; Turley et al. 1998; Kasim-Karakas et al. 2000). As discussed in section 2.8.3.1, the weight loss occurring with an ad libitum low-fat, high-carbohydrate diet often prevents fasting hypertriacylglycerolaemia. However, whether this is the case for the postprandial state as well has not been fully investigated. Another point for consideration is how long this weight loss lasts for. The two-year Women’s Trial Feasibility Study (Sheppard et al. 1991) showed that the intervention group that received intensive instruction in maintaining a low-fat, high-carbohydrate diet lost 3.0 kg in the first year. However, body weight gradually rose thereafter so that more than a third of the initial weight loss was lost by the end of the second year.

Epidemiologic studies have shown that some populations who consume very-low-fat diets have a low incidence of CHD, however, these populations also have a very low incidence of overweight and obesity and a high level of physical activity (Roberts
1988; Kagan et al. 1974). The levels of physical activity may be important in determining CHD risk during the consumption of low-fat diets. For example, physical inactivity among the USA population is now widespread so that one in four adults have sedentary lifestyles (NIH Consensus Conference 1996). At the same time, despite a decline in the intake of energy from fat (Stephen et al. 1995), obesity stands out as being increasingly prevalent in the United States (Grundy 1998). Thus, restricting fat intake in free living populations does not necessarily result in weight reduction, especially in affluent Western societies where the population is largely sedentary and the typical low-fat food items are often high in energy.

In conclusion, perhaps the most important and unambiguous generalisation that can be made from the findings of the studies described in this thesis is that daily moderate intensity exercise is sufficient to prevent the augmentation of postprandial lipaemia and accumulation of TRL particles attributable to a short-term low-fat, high-carbohydrate diet in men and women. High-carbohydrate diets are recommended by various scientific bodies for the prevention or treatment of CHD. However, whether these diets provide a net benefit with respect to CHD risk (Connor and Connor 1997) or actually increase the risk (Baum and Brown 2000) is an important public health issue. The present thesis demonstrated that the effects of high-carbohydrate diets on CHD risk factors, such as TAG metabolic capacity, could be strongly affected and beneficially altered by regular exercise. The programme of moderate exercise used in this thesis was acceptable to and generally enjoyed by the majority of subjects and it appears to be realistic for a large proportion of sedentary and maybe overweight people. Regular exercise may provide one means for allaying current anxieties about high-carbohydrate diets. However, studies of longer duration are necessary to evaluate the role and importance of exercise as an adjunct to dietary advice to the population to replace fat with carbohydrate.
REFERENCES


orphic, physical, dietary and biochemical characteristics. *Journal of Chronic Diseases* 27, 345-364.


Patsch JR, Karlin JB, Scott LW, Smith LC, Gotto AM. (1983) Inverse relationship between blood levels of high density lipoprotein subfraction 2 and the magnitude of
postprandial lipemia. *Proceedings of the National Academy of Science USA* 80, 1449-1453.


Patsch JR, Prasad S, Gotto AM, Patsch W. (1987) High density lipoprotein 2: Relationship of the plasma levels of this lipoprotein species to its composition, to the magnitude of postprandial lipemia, and to the activities of lipoprotein lipase and hepatic lipase. *Journal of Clinical Investigation* 80, 341-347.


Reaven GM. (1997) Do high-carbohydrate diets prevent the development or attenuate the manifestations (or both) of syndrome X? A viewpoint strongly against. *Current Opinion in Lipidology* 8, 23-27.


van Gent CM, Mirani-Oostdijk C, van Reine PH, Fröligh M. (1979) Influence of meal frequency on diurnal lipid, glucose and insulin levels in normal subjects on a high fat diet; comparison with data obtained on a high carbohydrate diet. *Journal of Clinical Investigation* 9, 443-446.


Weisgraber KH, Mahley RW, Kowal RC, Herz J, Goldstein JL, Brown MS. (1990) Apolipoprotein C-I modulates the interaction of apolipoprotein E with b-migrating very low density lipoproteins (b-VLDL) and inhibits binding to low density lipoprotein receptor-related protein. *Journal of Biological Chemistry* 265, 22453-22459.


West CE, Sullivan DR, Katan MB, Halferkamps IL, van der Torre h. (1990) Boys from populations with high-carbohydrate intake have higher fasting triglyceride levels than boys from populations with high-fat intake. *American Journal of Epidemiology* 131, 271-282.


APPENDICES

Appendix A: Information for volunteers, questionnaires, forms, food inventory, instructions for diet recording.

Appendix B: Plasma and TRL-TAG assay, serum insulin assay.
Influence of exercise on the hypertriglyceridaemic effect of a low-fat, high-carbohydrate diet: a study in the postprandial state.

BACKGROUND

Work in the Muscle Metabolism Research Group in this Department is looking at the effects of different types of diet and exercise on the body's ability to cope with fat contained in food. This capacity has important implications in determining one's risk of developing coronary heart disease. After eating a meal, blood vessel walls are exposed to particles carrying fat (triglycerides) from the food (exogenous triglycerides) as well as triglycerides produced from the body (endogenous triglycerides) that may facilitate the build-up of fatty deposits on the artery surface. High blood triglyceride responses to meals could accelerate this process.

A short-term high-carbohydrate diet has been shown to augment blood triglyceride responses to a meal possibly by promoting the production of endogenous triglycerides and/or by reducing the body's capacity to clear triglycerides from the circulation. Moderate exercise has been shown to reduce blood triglyceride responses to a meal, when participants are on a typical-UK diet, but the effect is unclear when participants are on a high-carbohydrate diet.

The first aim of this study is to compare the blood triglyceride response to a high fat meal following a 3-day high carbohydrate diet with that after a 3-day Western diet. The second, and more important, aim is to examine blood triglyceride responses after a 3-day high carbohydrate diet with participants also performing 30 minutes of exercise each day, to determine whether exercising while consuming a high carbohydrate diet provides a favourable alteration to triglyceride metabolism.

STUDY DESIGN

All volunteers will undertake a series of tests during the study. These tests involve the evaluation of:

- Fitness, by exercise tests.
- Body composition.
- Blood triglyceride responses to a test meal after 3 days on a typical-Western diet while refraining from exercise (Western intervention).
- Blood triglyceride responses to a test meal after 3 days on a high-carbohydrate diet while refraining from exercise (high-carbohydrate intervention).
- Blood triglyceride responses to a test meal after 3 days on a high-carbohydrate diet, with 30 minutes of treadmill jogging on each day (high-carbohydrate plus exercise intervention).
PRELIMINARY PROCEDURES

Before enrolling in the study, you will be asked to attend the laboratory for a preparatory session during which we will:

- Ask you to record everything you eat and drink for 2 weekdays and 1 weekend day.
- Measure your blood pressure.
- Discuss and complete with you confidential questionnaires regarding your health and physical activity.
- Provide an opportunity for you to ask questions.

We will also ask you to come to the laboratory before breakfast one morning so we can take a small blood sample to check the triglyceride and cholesterol levels in your blood.

EXPERIMENTAL PROCEDURES

A. Preliminary Exercise Tests

A submaximal and a maximal exercise test will be undertaken on the treadmill at Loughborough University. Heart rate will be monitored and recorded throughout using a heart rate monitor and expired air will be collected at intervals using a mouthpiece and respiratory valve.

- The first test will be sub-maximal and last for 16 mins. The speed will be increased at the end of each 4-min period. This test is designed to familiarise you with running on a treadmill.

- The second test will be maximal. The gradient will increase every three minutes and the test will end when you decide that you are able to run for just one further minute. This test is designed to determine your body's ability to use oxygen and enables us to find the correct speed for you to run at during the high-carbohydrate plus exercise intervention.

B. Body Composition

The amount and distribution of your body fat will be determined by measuring body girths and by using callipers to measure skin fold thickness at four different sites (a sophisticated version of "pinch an inch"). Your height and weight will also be recorded. You will need to wear only underclothing for these measurements, which will be made in private.

C. Diet Recording

You will be asked to weigh and record everything that you eat and drink for two weekdays and one weekend day. You will be given detailed instructions how to do this. According to your daily energy intake, the experimenters will prescribe a typical Western diet and a high-carbohydrate diet for you. You will be asked to follow the typical Western diet during
the Western intervention. You will be asked to follow the high-carbohydrate diet during
the high-carbohydrate and the high-carbohydrate plus exercise interventions.
We will provide you with a set of scales to do this easily.

D. Main Interventions

These will be conducted in random order.

- Western intervention - You will be asked to refrain from physical activity (other than
‘everyday activities’) and consume a prescribed typical Western diet for three days
before attending the lab for an oral fat tolerance test (described below).

- High-carbohydrate intervention - You will be asked to refrain from physical activity and
consume a prescribed high-carbohydrate diet for three days before attending the lab for
an oral fat tolerance test.

- High-carbohydrate plus exercise intervention – You will be asked to perform a 60-
minute bout of moderate exercise (treadmill walking) each day for 3 days while
following a high-carbohydrate diet. The next day you will attend the lab for an oral fat
tolerance test.

You will be asked to follow a prescribed diet for the three days prior each intervention and
to refrain from exercise and alcohol.

E. Blood Triglyceride Responses to a Test Meal (Oral fat tolerance test)

You will be asked to come to the laboratory after an overnight fast (i.e. having eaten
nothing for 12 hours) and eat a test meal consisting of cereal, bananas, apples, nuts,
sultanas, coconut, chocolate and cream. If you are allergic to nuts, they will be omitted
from the meal. In order to determine triglyceride concentrations in the blood, we will take
small blood samples in the fasted state and at intervals over a period of 6 hours after the
meal. About 450 ml of blood will be removed in total over the three trials (about the
volume withdrawn if one volunteers to donate blood). To minimise any discomfort the
samples will be obtained via a cannula (tiny plastic tube) placed (under local anaesthetic)
in a forearm vein. Just before the meal and hourly for the next 6 hours we will take
expired air samples. For 6 hours following the meal, you will not be able to eat or drink
anything, except water. You will just be resting, watching TV, reading or working. After
this time we will provide you with a meal and then you can go home.

These tests will be done at your convenience.

HOW MUCH TIME WILL THE TESTS TAKE?

- Fitness tests: For the exercise test at the University you will be in the laboratory for no
more than 40 minutes on each occasion.

- Body composition: The procedure requires only a few minutes and will take place on
the same visit as one of the exercise tests.
• High-carbohydrate plus exercise intervention: The treadmill jogging will take 30 minutes each of the three days.

• Blood triglyceride responses to a fat meal: These tests will take place over a period of about 7 hours.

POSSIBLE RISKS AND DISCOMFORT

• Exercise testing will be at sub-maximal and maximal level and the possibility exists that, very occasionally, certain changes may occur during or shortly after the tests. They include abnormal blood pressure, fainting or a change in the normal rhythm of the heartbeat.

• Blood sampling via the cannula may cause minor bruising, an inflammation of the vein or haematoma (a small accumulation of blood under the skin). There is also a exceedingly small risk of a tiny piece of plastic or an air bubble entering the bloodstream if the cannula is incorrectly placed. Good practice, however, minimises this risk and only experienced and trained staff will deal with blood sampling.

The preliminary procedures before the start of the study are designed to minimise possible risks. All tests will be contacted and closely monitored by trained and experienced staff and in addition, only those at little risk from the procedures will be accepted into the study.

BENEFITS OF THE STUDY

The findings will be published in the scientific and medical literature so that understanding of the way in which diet and exercise influences the risk of heart disease can be increased.

We will provide you with feedback about the group findings and also about your own results and would be delighted to explain results and discuss the implications with you.

CONFIDENTIALITY

Although information will be stored on computer, each subject will be entered as a number rather than by name and will not be identifiable. This is in accordance with the Data Protection Act.

Any questions about the procedures used in this study are encouraged. If you have any doubts or questions, please ask for further explanations by contacting Christina Koutsari on 01509 228183 (Office), 222277 (Home), C.Koutsari@lboro.ac.uk (e-mail) or Prof. Adrianne Hardman on 01509 223265.
APPENDIX A2
STATEMENT OF INFORMED CONSENT

LOUGHBOROUGH UNIVERSITY, DEPARTMENT OF PHYSICAL EDUCATION, SPORTS SCIENCE AND RECREATION MANAGEMENT

Influence of exercise on the hypertriglyceridaemic effect of a low-fat, high-carbohydrate diet: a study in the postprandial state.

Statement of informed consent:

Your permission to take part in this study is voluntary. You are free to deny consent or to withdraw from the study at any point and without explanation, if you so desire.

I have read the information regarding this study and had the opportunity to ask questions of the investigators. I understand the procedures involved and consent to participate in this study.

Signature of subject: ___________________________ Date: ____________

Signature of witness: ___________________________
APPENDIX A3
HEALTH HISTORY QUESTIONNAIRE FOR STUDY VOLUNTEERS
CONFIDENTIAL

NAME

DATE

DATE OF BIRTH

ADDRESS

TELEPHONE

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

Please complete this brief questionnaire to confirm fitness to participate:

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

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

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

If YES to any question, please describe briefly if you wish (e.g. to confirm problem was/is short-lived, insignificant or well controlled.) .......................................................................................................................................

5. Additional questions for female participants
   (a) Are you taking hormone replacement therapy?......... Yes ☐ No ☐
   (b) For how long have you been on menopause? .......... Yes ☐ No ☐

Name and address of GP .........................................................

Thank you for your co-operation
APPENDIX A4
PHYSICAL ACTIVITY QUESTIONNAIRE

During one week, how many times on average do you do the following kinds of exercise for more than 15 minutes?

(a) **Strenuous Exercise (heart beats rapidly)**
   For example; running, jogging, squash, hockey, football, basketball, vigorous swimming, vigorous long distance cycling.
   
   _________ times per week.

(b) **Moderate Exercise (not exhausting)**
   For example; fast walking, tennis, easy cycling, badminton, easy swimming, dancing.
   
   _________ times per week.

(c) **Mild Exercise (minimal effort)**
   For example; yoga, archery, fishing, bowling, golf, easy walking.
   
   _________ times per week.
APPENDIX A5
FOOD INVENTORY FORM

<table>
<thead>
<tr>
<th>Name</th>
<th>Date</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>1. Time/Place</th>
<th>2. Description of food/drink</th>
<th>3. Weight of food/drink (g)</th>
<th>4. Weight of leftovers (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
It is important that you weigh and record everything that you eat and drink (except water) for two week days and one weekend day.

Please (i) start a separate page for each day
(ii) start a separate line for each item.

Column 1
Record meal and time and place of eating.

Column 2
Describe each item as accurately as possible, starting where relevant:
(i) type and brand
(ii) whether food is fresh, dried, canned, frozen, salted, smoked, etc.
(iii) whether food is cooked, if so give method of cooking e.g. fried, baked, etc.

Column 3
Record the weight of each item preferably after cooking (fried chicken-lean meat, baked potatoes, lasagne, chicken curry, boiled rice etc.):
(i) place scales on a level surface
(ii) place plate or container on top of scales
(iii) press ‘ON/Reset’ button to turn on scales
(iv) once zero appears, add first item of food
(v) record weight displayed
(vi) press reset button before weighing next item

Record weights in grams. If this is not possible, record weights in household measures (e.g. sugar or jam in teaspoons, stating whether level, rounded, or heaped).

If you are going to cook a food following a special recipe (very different from the common one in terms of the kind and quantity of the ingredients):
(i) weigh and record each of the ingredients raw (do not forget to weigh and record the water)
(ii) weigh and record the whole food cooked
(iii) weigh and record your portion

e.g.: meat and tomato (pasta) sauce:
Raw ingredients: 200 gr lean beef mince
350 g peeled plum tomatoes in tomato juice (no sugar)
50 g green peppers
15 g olive oil
30 g red onion
10 g parsley
20 g water
10 g sugar
3 g salt, 2 g pepper

Whole food cooked: 550 g
Your portion: 230 g
Column 4
Record the weight of any leftovers, such as food remaining on plate, weight of container in which food has been weighed, apple cores, etc.

If food consists of several items, please list each on a separate line i.e. instead of writing 'one cheese sandwich', record separately the weights of bread, margarine, cheese, etc.

Eating out: If you eat foods away from home, please do not forget to record these as well. Take your diary and scales with you where it is possible. If this is too inconvenient, just record the types of food eaten with an estimated weight—but please specify when a weight has been estimated.

Please try to be as accurate as possible and try to choose fairly representative days to record your food intake. For instance do not record days when you are involved in activities that would alter your normal diet (e.g. illness, holiday).

An example is shown overleaf.
## FOOD INVENTORY

<table>
<thead>
<tr>
<th>1. Time/Place</th>
<th>2. Description of food/drink</th>
<th>3. Weight of food/drink (g)</th>
<th>4. Weight of leftovers (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast</td>
<td>Corn flakes (Kellogs)</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>8:30am</td>
<td>Milk (Sainsbury’s virtually fat-free)</td>
<td>218</td>
<td></td>
</tr>
<tr>
<td>Home</td>
<td>White bread (Mothers Pride, toasted)</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flora spread, 70 % fat, PUFA</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Robertson strawberry jam</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Milk (whole pasteurised)</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Coffee (instant)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Lunch</td>
<td>White bread</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>1:00pm</td>
<td>Cheese (Cheddar, extra mature)</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Pub</td>
<td>Butter</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ham</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Banana</td>
<td>197</td>
<td>32 (skin)</td>
</tr>
<tr>
<td></td>
<td>Orange Tango</td>
<td>can</td>
<td></td>
</tr>
<tr>
<td>Snack</td>
<td>Coffee (instant)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3:30pm</td>
<td>Milk (semi-skimmed, pasteurised)</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>office</td>
<td>Mars bar</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apple (Granny Smith)</td>
<td>76</td>
<td>10 (core)</td>
</tr>
<tr>
<td>Dinner</td>
<td>Turkey fillet (grilled)</td>
<td>123</td>
<td></td>
</tr>
<tr>
<td>7:00pm</td>
<td>Old potatoes, boiled</td>
<td>228</td>
<td>42 (leftover)</td>
</tr>
<tr>
<td>Home</td>
<td>Peas (Birds Eye, frozen, boiled)</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heinz tomato ketchup</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yogurt (Müller thick and creamy)</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td>Snack</td>
<td>Potato crisps (Walkers)</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>9:00pm</td>
<td>Sorbet, lemon</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>Home</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX B1
DETERMINATION OF PLASMA AND TRL-TAG CONCENTRATION WITH CORRECTION FOR FREE GLYCEROL

TAG concentration is chapters 5 and 6 were determined using the method by Humphreys et al. (1990) with correction for free glycerol.

Test principle

1. TAG $\xrightarrow{\text{Lipoprotein lipase}}$ Glycerol + Fatty Acids

2. Glycerol + ATP $\xrightarrow{\text{Glycerokinase}}$ Glycerol-3-phosphate + ADP

3. Glycerol-3-phosphate + NAD$^+$ $\xrightarrow{\text{Glycerol-3-phosphate dehydrogenase}}$ Dihydroxyacetone phosphate + NADH + H$^+$

4. NADH + H$^+$ + INT $\xrightarrow{\text{Diaphorase}}$ NAD + formazan

TAG was hydrolysed by lipoprotein lipase to yield glycerol and fatty acids. The glycerol was then phosphorylated (glycerokinase) to glycerol-3-phosphate. This was oxidised with glycerol-3-phosphate dehydrogenase to dihydroxyacetone phosphate and NADH + H$^+$ and finally, a coloured formazan was formed by reduction of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT).

The coloured formazan end product shows an absorbance maximum at 500 nm. The increase in absorbance at 500 nm is, therefore, directly proportional to the TAG concentration of the sample. For the determination of free glycerol, the reaction was repeated with omission of lipoprotein lipase (step 1). TAG concentration was calculated from the difference between total and free glycerol.

Reagents

Trizma base (Sigma Diagnostics, Poole, UK), cholic acid, sodium salt (Sigma Diagnostics, Poole, UK), albumin, bovine, fatty acid free, 99% (Sigma Diagnostics, Poole, UK), Triton X-100 (Sigma Diagnostics, Poole, UK), magnesium chloride,
hexahydrate (Merck, Poole, UK), NAD, grade II (Roche Diagnostics Ltd., Lewes, UK),
adenosine-5-triphosphate, di-sodium salt (Roche Diagnostics Ltd., Lewes, UK),
glycerokinase, 5 mg/mL (Roche Diagnostics Ltd., Lewes, UK), glycerol-3-phosphate
dehydrogenase, 10 mg/mL (Roche Diagnostics Ltd., Lewes, UK), diaphorase, 200 U
(Roche Diagnostics Ltd., Lewes, UK), NADH:dye oxidoreductase (Roche Diagnostics
Ltd., Lewes, UK), lipase, type XIII, 1000 U (Sigma Diagnostics, Poole, UK), of 2-(4-
iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT) (Merck, Poole, UK),
Na₂HPO₄ (anhydrous) and Na₂HPO₄·2H₂O (Merck, Poole, UK).

Solutions

0.1 mol/L Trizma, pH 7.9
For 500 mL, the following amounts were weighed:
6.055 g Trizma base
0.75 g sodium cholate
5 g albumin, bovine, fatty acid free
These were dissolve in about 100 mL of distilled water and then 0.75 g Triton X-100 was
added. They were mixed until dissolved. The pH was adjusted to 7.9 with approximately
5 mol/L HCl. Distilled water was then added to make up to 500 mL. The final solution
was stored at 4°C.

0.1 mol/L sodium phosphate buffer, pH 7.0
For 500 mL, the following amounts were weighed:
4.33 g Na₂HPO₄ (anhydrous) or 5.43 g Na₂HPO₄·2H₂O
3.04 g Na₂HPO₄·2H₂O
These were dissolved in about 400 mL distilled water. The pH was checked (7.0) and
adjusted if necessary. Distilled water was then added to make up to 500 mL. The final
solution was stored at 4°C.

0.1 mol/L magnesium chloride
2.03 g of MgCl₂·6H₂O was dissolved into 100 mL distilled water. The solution was stored
at 4°C.
1 % NAD
100 mg NAD was dissolved in distilled water (to make up to 10 mL). The solution was stored in aliquots at -20°C.

0.1 mol/L ATP
603 mg ATP was dissolved in distilled water (to make up to 10 mL). The solution was stored in aliquots at -20°C.

12 mmol/L INT
60 mg INT was dissolved in distilled water (to make up to 10 mL). The solution was stored in aliquots at 4°C.

Diaphorase
200 U diaphorase was dissolved in 1 mL of 0.1 mol/L sodium phosphate buffer pH 7.0. The solution was stored at 4°C in the bottle that diaphorase was supplied in (stable for 6 weeks).

Lipase
1000 U lipase was dissolved in 1.4 mL of 0.1 mol/L sodium phosphate buffer pH 7.0. The solution was stored at 4°C in the bottle that lipase was supplied in.

Glycerokinase and glycerol-3-phosphate dehydrogenase
These were used as supplied after mixing well. They were stored at 4°C.

Working reagent for free glycerol assay
The following proportions were mixed:
10 mL 0.1 mol/L Trizma pH 7.9
1 mL 0.1 mol/L MgCl₂·6H₂O
1 mL 1 % NAD
0.5 mL 0.1 mol/L ATP
20 μL glycerokinase
100 μL glycerol-3-phosphate dehydrogenase
200 μL diaphorase
For each 1 mL of the above working reagent, 100 μL 12 mmol/L INT was added just before the analysis (the INT has to be added as late as possible because it turns pink in the light). The working reagent was ready for use.

**Working reagent for total glycerol assay**

The following proportions were mixed:

- 10 mL 0.1 mol/L Trizma pH 7.9
- 1 mL 0.1 mol/L MgCl₂·6H₂O
- 1 mL 1 % NAD
- 0.5 mL 0.1 mol/L ATP
- 20 μL glycerokinase
- 100 μL glycerol-3-phosphate dehydrogenase
- 200 μL diaphorase
- 100 μL lipase

For each 1 mL of the above working reagent, 100 μL 12 mmol/L INT was added just before the analysis. The working reagent was ready for use.

Samples were analysed in duplicate for total and free glycerol on an automated analyser (Cobas Mira Plus, Roche Diagnostic Systems, Hertfordshire, UK). For calibration, three calibrators were prepared from a commercially available 2290 μmol/L glycerol solution (Roche Diagnostics Ltd., Lewes, UK). This calibrator was serially diluted with distilled water to produce calibrators appropriate for the assay range. For plasma and TRL free glycerol assay, these were 143 μmol/L, 286 μmol/L and 572 μmol/L. For plasma total glycerol assay, calibrators of 572 μmol/L, 1145 μmol/L and 2290 μmol/L were used. Corresponding values for TRL total glycerol assay were 286 μmol/L, 572 μmol/L and 1145 μmol/L. For plasma total and free glycerol, 4 μL of sample as required for each analysis. For TRL total and free glycerol assay, 20 μL and 30 μL were required, respectively. For all assays, 110 μL of working reagent was required per measurement.
APPENDIX B2
DETERMINATION OF SERUM INSULIN CONCENTRATION

Concentrations of serum insulin were determined using a solid-phase $^{125}$Iodine radioimmunoassay (COAT-A-COUNT; Diagnostic Products, Los Angeles, CA, USA). Radioactivity was measured using an automated gamma counting system (Cobra II, Packard Instrument, Downers Grove, IL).

Test principle
In this procedure, $^{125}$Iodine labelled insulin competes with insulin in the sample for sites on insulin-specific antibodies which are immobilised on the wall of a polypropylene tube. After an incubation period, the antibody bound fraction is achieved by decanting the supernatent. The radioactivity of the tube is then measured, with the counts being inversely related to the amount of insulin present in the serum sample. Serum insulin concentration is then determined by comparing the counts to a standard curve.

Materials and reagents
- Plain (uncoated) polypropylene tubes for the measurement of total and non-specific binding counts.
- Green polypropylene tubes coated with antibodies to insulin.
- A concentrated solution of $^{125}$Iodine labelled insulin, which was diluted with 100 mL distilled water.
- Lyophilised processed human calibrators were supplied with the kit at insulin concentrations of 0, 5, 15, 50, 100, 200 and 400 μIU/mL. These were reconstituted with distilled water at least 30 minutes before use.
- A three-level, human-serum based, quality control was used (CON6, Diagnostic Products, Los Angeles, CA, USA).

Procedure
1. 200 μL of the 0 μIU/mL calibrator was dispensed into the plain non-specific binding tubes.
2. 200 μL of each calibrator, serum sample or quality control was dispensed directly to the bottom of each antibody-coated tube.
3. 1.0 mL of the $^{125}$Iodine labelled insulin solution was added to the plain total count tubes and each antibody-coated tube, within 40 minutes of dispensing the calibrator/sample/quality control.

4. Tubes were vortexed and allowed to incubate for 18 to 24 hours at room temperature.

5. After this incubation period, the contents of each tube (except the total count tubes) were decanted using a foam decanting rack. The tubes were allowed to drain for about 5 minutes and were then struck sharply on absorbant paper to remove residual droplets remaining in the tubes.

6. The radioactivity of each tube was then counted for 1 minute in the gamma counter.

7. Insulin concentrations were determined from a spline fit calibration curve calculated from the values of the calibrators.