The influence of high carbohydrate diets and glycaemic index on metabolic risk parameters for coronary heart disease.

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Summary

This thesis describes a series of studies investigating the relationship between diet, and in particular carbohydrate intake, and risk factors for type 2 diabetes and coronary heart disease (CHD).

The first study investigated the effect of advice to increase carbohydrate intake as part of dietary advice to follow the dietary guidelines on metabolic risk factors for CHD in postmenopausal women (Chapter 3).

The results showed that subjects appeared to have followed the dietary advice given as they reported significantly reducing their total daily energy ($P = 0.011$), fat ($P = 0.008$) and non-milk extrinsic sugar (NMES) intake ($P = 0.015$), and significantly increasing their total carbohydrate ($P = 0.026$), starch ($P = 0.013$) and non-starch polysaccharide (NSP) intake ($P = 0.050$). Subjects also significantly increased their dietary glycaemic index (GI) ($P = 0.011$). There was a significant reduction in body mass index (BMI) ($P = 0.014$), and an adverse effect on fasting plasma lipids including an increase in fasting TAG ($P = 0.014$), and a decrease in HDL cholesterol concentrations ($P = 0.021$). Subjects reported increasing their consumption of fruit and vegetables, and there was a significant increase in the 'antioxidant power' of plasma ($P = 0.007$). This appears to have mostly been associated with an increase in fruit intake.

Correlation analyses showed that simple sugars appeared to have a more adverse effect on plasma lipids than starch. From this a decision was made to study the relationships between GI and plasma lipids and other metabolic risk factors in data that had already been collected.

The main findings of this case control study on offspring of patients of type 2 diabetes (offspring) and control subjects (Chapter 4) showed that there were no differences in habitual dietary intake, GI or GL between the groups. Offspring were found to demonstrate many of the features of the metabolic syndrome as they had greater levels of adiposity and female offspring had significantly higher waist to hip

ratio ($P = 0.036$), waist circumference ($P = 0.063$) and BMI ($P = 0.083$) compared with female control subjects. Offspring were significantly more insulin resistant compared with control subjects with significantly higher fasting insulin ($P = 0.049$) and higher $HOMA_{IR}$ ($P = 0.052$) and significantly lower HDL cholesterol concentrations ($P = 0.011$).

However, dietary GI and GL were not found to be directly associated with any of the metabolic parameters measured in the study, but GI was positively correlated with waist circumference ($P = 0.039$) and waist to hip ratio ($P = 0.043$), and measures of adiposity were significantly correlated with many of the metabolic parameters measured in the study. Thus, while the glycaemic quality of the diet did not appear to directly influence metabolic risk factors, the results do support the idea that they influence metabolic risk factors through their effect on adiposity, and in particular central adiposity.

There are very few intervention studies that have been carried out in healthy individuals, of either short or longer-term. Thus, to find out if the benefits of reducing fat intake (i.e. cholesterol lowering) could be maintained, and the adverse effects associated with increasing carbohydrate intakes could be avoided if dietary fat was replaced with carbohydrates of low GI, a high and low GI a short-term experimental intervention study on healthy individuals was carried out (Chapter 5).

The results of this randomised crossover study showed that the low GI diet has some beneficial effects in that it reduced total ($P = 0.029$) and LDL cholesterol. Both high and low GI intervention diets had adverse effects on TAG and HDL cholesterol concentrations but this was probably due to the fact that the diets were high in carbohydrate (70% energy intake). TAG concentrations were found to be higher after the low GI diet ($P = 0.004$), which was not an expected finding but could possibly be explained by the fact that the low GI diet was higher in sugars compared with the high GI diet ($P = 0.001$). These results are interesting and suggest that the overall carbohydrate and sugar content of the diet have a more important influence on plasma lipids and other metabolic parameters than the glycaemic quality of the diet.

In these studies, there were some very important beneficial effects found (reduction in body weight, increase in 'antioxidant power', reduction in total and LDL cholesterol) following dietary advice. However, this same advice led to adverse effects on TAG and HDL cholesterol which are associated with in increased risk of CHD. In the first study, worse effects were found when women increased their simple sugar intake at the expense of starch intake. Also low GI diet in the third study seemed to have a more adverse effect on TAG and HDL cholesterol compared with the high GI diet. However, the low GI diet was higher in sugar compared with the high GI diet. The fact that these carefully planned low GI diet was high in sugar highlights an interesting problem with the concept of GI. Some foods which are high in sugar especially fructose have a low GI but still have an adverse effects on lipids. This fact is not generally known and makes it difficult for the general public to follow the dietary guidelines safely. For this reason it may be safer to advise the public to consume more slowly digestible carbohydrates (such as wholegrain cereals, pulses) rather than low GI foods which could still contain a high proportion of sugar which could mask the positive effects of the low GI diet on lipids. Overall, the results from this thesis highlight the need for more research to develop safer and more appropriate dietary guidelines which can be easily and clearly communicated to the general public.

Dedication

Thanks to Allah for creating the possibility for me to study for this PhD.

Enduring love for Iranian soldiers who died and dedicated their life for their country; I dedicate this thesis to their parents who wished their son to be with them and to have had the life opportunities that I have had.

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List of Abbreviations

24-hr recall	24-hour recall
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
AUC	Area under curve
BMI	Body mass index
BMR	Basal metabolic rate
BPG	Biphosphoglycerate
CATP	Cholesterolacyl transfer protein
CHD	Coronary Heart Disease
CI	Confidence interval
CoA	Coenzyme A
CRP	C-reactive protein
CV	Coefficient of variation
CVD	Cardiovascular disease
E1	Oestrone
E2	Estradiol
E3	Estriol
EDTA	Ethylenediaminetetra-acetate
EI	Energy intake
ELISA	Enzyme linked immunosorbent assay
FAD	Flavinadenine dinucleotide
FADH2	Reduced Flavinadenine dinucleotide
FAO	Food and Agriculture Organization
FFA	Free fatty acids
FFQ	Food frequency questionnaire
FRAP	Ferric reducing ability of plasma
G-6-P	Glucose-6-phosphate
GI	Glycaemic Index
GI tract	Gastrointestinal tract
GL	Glycaemic Load
Gly	Glucose
HC	High carbohydrate

HCL	Hydrochloric acid
HDL	High density lipoprotein
$HOMA_{IR}$	Homestatic model assessment
HRT	Hormone replacement therapy
IL-6	Interleukin-6
IRI	Insulin resistance index
ISI	Insulin sensitivity index
ITT	Insulin tolerance test
IVGTT	Intravenous glucose tolerance test
Kcal	Kilocalorie
Kj	Kilojoule
LCAT	Lecithincholesterolacyl transferase
LDL	Low density lipoprotein
LF	Low fat
LPL	Lipoprotein lipase
MRFIT	Multiple Risk Factor Intervention Trial
MONICA	Monitoring trends and determinants of Cardiovascular diseases
MUAC	Mid-upper arm circumference
MUFA	Monounsaturated fatty acids
NAD	Nicotinamide Adenine Dinucleotide
NADH	Reduced Nicotinamide Adenine Dinucleotide
NEFA	Non-esterified fatty acid
NMES	Non-milk extrinsic sugar
NSP	Non-starch polysaccharides
OGTT	Oral glucose tolerance test
OR	Odds ratio
PFK	Phosphofructokinase
PUFA	Polyunsaturated fatty acids
RIA	Radioimmunoassay
rpm	Revolutions per minute
RR	Relative risk
SD	Standard deviation
SF	Saturated fatty acids

Chapter 1

Introduction

1.1 Introduction

This thesis describes a series of studies investigating the relationship between diet and risk factors for type 2 diabetes and coronary heart disease (CHD).

Chronic imbalance of normal metabolism is a cause of a number of complex diseases such as type 2 diabetes mellitus and atherosclerosis (Whitefield *et al.* 2004). Results of the WHO project, Monitoring Trends and Determinants of Cardiovascular Disease (MONICA) in 21 European countries, indicate that CHD incidence is higher in Northern, Central and Eastern regions than in Southern and Western Europe (WHO Regional Office for Europe, 2002). CHD causes more than 100,000 deaths per year in the UK and Scotland is number one in the world for this disorder (The Scottish Office, 2000). In the UK it has been estimated that 2.65 million men and women have or have had CHD (either angina or heart attack). The UK is experiencing a slow decline in coronary mortality rate (Yarnell *et al.* 2003). In Scotland, the prevalence of CVD was around 23.5% in 1998 and there was no change since 1995. Results of the 1998 Scottish health survey showed that the Scottish population, particularly women, were more likely to have ischemic heart disease (IHD) or stroke than people in England (The Scottish Office, 2000).

Recent data show that mortality from CHD in Scotland is higher than the UK average and is the highest in the western world (Tunstall *et al.* 1999). From the 21 countries in the MONICA study which monitored trends and determinants of cardiovascular disease, Glasgow had the highest mortality from CHD (Tunstall *et al.* 1999; Richards *et al.* 2002). Large-scale epidemiological studies have revealed that hypercholesterolaemia and hypertriglyceridemia are major risk factors for CHD. A meta-analysis of 17 population based studies showed a 76% increase in

cardiovascular disease risk in women and a 31% increase in men associated with a one mmol/L increase in plasma triacylglycerol (TAG) levels (Austin, 1999). Both endogenous and exogenous TAG contribute to the circulating level of TAG and dietary carbohydrate is one of the most important precursors for plasma TAG in the human diet.

Current dietary guidelines recommend a high carbohydrate diet (>55% energy from carbohydrate), but several studies have indicated adverse effects of such a diet on plasma lipids with an increase in TAG and a decrease in HDL cholesterol (Shah *et al.* 1994; Kasim-karakas *et al.* 1997; Jeppesen *et al.* 1997; Kasim-karakas et al, 2000; Hudgins *et al.* 2000; Mittendorfer and Sidossis, 2001 and Bunyard *et al.*, 2002). It may be that the type of carbohydrate is important, for example its digestibility, the glycaemic index (GI) of the food and the physiological state of the subject. These factors are investigated in the studies presented in this thesis.

Dietary carbohydrate inake has increased since 1963 by 126g per day in USA. The type of carbohydrate has also changed. This increase in consumed carbohydrate includes an increase in fructose corn syrup intake (10% of total energy intake) (Gross *et al.* 2004). Consumption of fructose has increased because soft drinks and some foods have been sweetened with high fructose corn syrup by manufacturers (Bray *et al.* 2004). There have been several studies that suggest fructose may be an independent risk factor for high TAG (Gross *et al.* 2004). This evidence will be discussed later.

A high carbohydrate diet is also usually low in fat. Results of the National survey of energy and macronutrient intakes in the UK have shown reductions in per capita energy and fat intakes since 1975, while carbohydrate intakes has been steady over

the period (Department of Agriculture, 2004). Glucose and fatty acids are the major oxidative fuels in human metabolism and account for approximately 80% of oxidative metabolism. Their classification and metabolism will be considered separately and their interactions discussed. Before the relationships between diet and disease are discussed in this chapter, normal metabolism will be considered briefly and the effects of diet on metabolism discussed in more detail.

There are a number of different study designs used in the studies that will be discussed in this thesis and that have been used in the research described in this thesis. Different designs are used for different purposes and there are a number of strengths and weaknesses associated with each (Gibney *et al.* 2004). Epidemiological studies evaluate the association between exposures such as diet or other characteristics with disease risk or risk factors and try to explain differences seen. Observation, epidemiological studies include case control, cross-sectional and cohort studies. Case-control studies examine whether persons with a disease have the same diet as individuals without and, assume that the measure of dietary exposure (ie. plasma lipids) has not been influenced by the disease process. The strengths of case control studies is that are relatively inexpensive to carry out as they require smaller number of subjects compared with prospective studies and for this reason are very good for studying rare diseases. In cross-sectional studies, diseases or risk factors and exposure such as diet are measured at the same time and this is a weak design for assessing causal relationships. Cohort studies can be either retrospective or prospective and assess whether persons with, for example, a high dietary fat intake develop the disease or die from the disease more often than those who do not have a high fat intake. Cohort studies have a number of strengths in that the exposure comes before the development of the disease and they do not rely on subjects' memory of what they had eaten in the past. One major problem with observational studies in general is the presence of confounding factors. Confounding happens when one the component of diet is associated with another dietary factor that is

related to the disease or risk factor that you are interested in. Intervention studies are superior to observation studies in that a researcher is able to test for an independent effect of one nutrient and to hold the others constant. A randomised controlled trial is considered to be the best method for a number of reasons including: control or untreated group, the subjects or patients are randomised into treatment groups which reduces the likelihood or chance findings, and they are blinded which controls for the placebo effect. Sometimes RCTs can have a cross-over design which means that both groups follow both treatments or intervention in turn, usually with a wash-out period in between. However, RCTs are expensive to carry out and the results depend heavily on the compliance of subjects. Quasi-experimental studies are often carried out where a RCT is not always possible, such as in studies where dietary advice is given and randomisation is not always possible. These strengths of these type of studies is that they do have a before and after measurement and can be conducted in free-living subjects which means the intervention can be more relevant to real life situation. However, this design also has limitations in that it also depends on compliance which is difficult to control in free living subjects and can the results can be influenced by non intervention effects for example, sometimes when subjects are given dietary advice they also change their physical activity habits (Gibney *et al.* 2004).

1.2 Carbohydrate

The single most important source of food energy in the world is dietary carbohydrate (FAO/WHO 1997). Depending on the geographic area and individual economic conditions, carbohydrates comprise 40 to 80 percent of total food energy intake. In the UK, 50% of daily energy comes from fat (Englyst *et al.* 1992). The Food and Agriculture organization (FAO) and World Health Organization (WHO) Expert Consultation (FAO/WHO 1997; 2003), which provide evidence to improve nutritional knowledge for developing and developed countries, include the widely accepted definition and classification for dietary carbohydrate described below.

Starch is the primary product of photosynthesis by which plants use energy from sunlight to synthesise carbohydrate from carbon dioxide and water. Sugars have been divided into intrinsic and non-intrinsic sugars depending on their situation in food (see later). The major sources of carbohydrates in the diet are:

- Cereals,
- Root crops,
- Sugar crops,
- Pulses,
- Vegetables,
- Fruits,
- Milk products (Department of Health, 1991).

1.2.1 Classification

There are various types of classification for carbohydrates including structural (degree of polymerisation according to biochemical structure) and physiological classifications according to the fate and digestibility of the carbohydrate. This latter type of classification includes the glycaemic index and amount of rapidly available carbohydrate, and is considered more relevant for the evaluation of the physiological effects of dietary carbohydrate. The fate and effects of dietary carbohydrates depend on the nature of the carbohydrate, the matrix of the food and the biochemical structure of the consumed meal (Laville, 2004). Carbohydrates are more conventionally classified according to their structure and the molecular size (i.e. the number of sugar units) into:

- Monosaccharides containing one sugar unit (such as glucose, galactose, and fructose)
- Disaccharides containing 2 sugar units (e.g. sucrose, lactose, trehalose)

- Oligosaccharides containing 3-9 sugar units (e.g. maltodextrins, raffinose, stachyose, fructo-oligosaccharides)
- Polysaccharides containing >9 sugar units (e.g. amylose, amylopectin, modified starches, and non-starch polysaccharides such as cellulose, hemicellulose, pectin, hydrocolloids) (Gibney *et al.* 2002).

Table 1.1 Various types of dietary carbohydrate and their digestion products

Class	Example	DP	Site of digestion	Produced metabolites
Monosaccharides	Glucose	1	Small intestine	Glucose
	Fructose	1	Small intestine	Fructose
Dissaccharides	Lactose	2	Small intestine	Glucose+Fructose
	Sucrose	2	Small intestine	Glucose+Galactose
Oligosaccharides	Raffinose	3	Large intestine	SCFA, acetate,
	Inulin	3-9		propionate and
	Pyrodextrins	3-9		butyrate
Polysaccharides	Starches	>9	Small intestine§	Glucose via maltose, maltotriose and dextrins
	NSP	>9	Large bowel	SCFA

*** Source: Gibney *et al.* 2002, DP: Degree of polymerisation, NSP: Non starch polysaccharides, SCFA: Short chain fatty acids, § Some starch escapes small intestine digestion and in all these conditions the carbohydrate enters the large bowel and is fermented to SCFA by colonic bacteria.**

1.2.2 Carbohydrate digestion

Table 1.2 summarises the process of digestion and absorption of dietary carbohydrates and fats in different parts of the gastrointestinal (GI) tract in humans.

Table 1.2 The process of digestion and absorption of carbohydrates and fats in different parts of the gastrointestinal tract

	Function	Enzyme/Compound	Substrate	Products
Mouth	Breaking up foods through chewing Starch digestion	Salivary α-amylase	Hydrolysis of α-1,4-glucosidic linkage of starch and glycogen	Maltose, maltotriose and dextrins
	Limited lipid digestion	Lingual lipase	Long chain triglycerides	fatty acids and 2-monoglycerides
Stomach	Disruption and liquidation of food particles Inactivation of α-amylase by gastric acid but can have continued hydrolysis of starch (up to 50%) if food buffers acid.			
	Emulsification and degradation of fat (≈10%)	Gastric lipase	Long chain triglycerides	fatty acids and 2-monoglycerides
Small intestine	Hydrolysis of: - starch	Pancreatic α-amylase	Hydrolysis of: α-1,4-glucosidic linkage	α limit dextrins, maltotriose, and maltose
	- α limit dextrins	Brush border α dextrinase	α-1,6-glucosidic linkage	
	- maltose	Brush border maltase	α-1,4-glucosidic linkage	Glucose
	- sucrose	Brush border sucrase	α-1,2-glucosidic linkage	Glucose and fructose
	- lactose	Brush border lactase	β-1,4-glucosidic linkage	Glucose and galactose
	- trehalose	Brush border trehalase	Trehalose	Glucose
	Absorption	Active transport	Glucose and galactose	Glucose
	Absorption of fructose	Faciliated diffusion	fructose	

Table 1.2 Continued The process of digestion and absorption of carbohydrates and fats in different parts of the gastrointestinal tract

	Function	Enzyme / Process	Substrate	Products
	Fat emulsification	Bile acids	TAG	Micelles
	Hydrolysis of fat	Pancreatic lipase, phospholipase and cholesterol ester hydrolase	and phospholipids	Short-, medium- and long chain fatty acids, monoglycerides
Gut cells	Absorption of short- and medium-chain fatty acids and glycerol into portal vein	Diffusion or specific carriers		
	Absorption of long-chain fatty acids	Diffusion		Chylomicrons into lacteals and lymphatic system
	Chylomicron formation in the cells			
	Reabsorption of bile acids	Active transport		
Colon	Fermentation of indigested carbohydrates by colonic bacteria		Resistant starch, raffinose, inulin, dietary fibre	Short-chain fatty acids (e.g. acetic, butyric and propionic acids), gases (e.g. CO_2, methane, hydrogen) rapidly absorbed
	Increased stool output			
	Bacterial metabolism of bile acids		Primary bile acids	Secondary bile acids

1.2.3 Carbohydrate metabolism

Carbohydrates are metabolised by several pathways with different functions. These pathways usually start with glucose, although other sugars can enter through appropriate intermediates. The released energy is trapped in the form of ATP for use by energy-consuming activities of the cell. Glucose uptake by the tissues is controlled by facilitated diffusion tissue specific transporters (GLUTs) which are physiologically expressed and this leads to differential uptake of glucose in different organs (Grover-McKay *et al.* 1999).

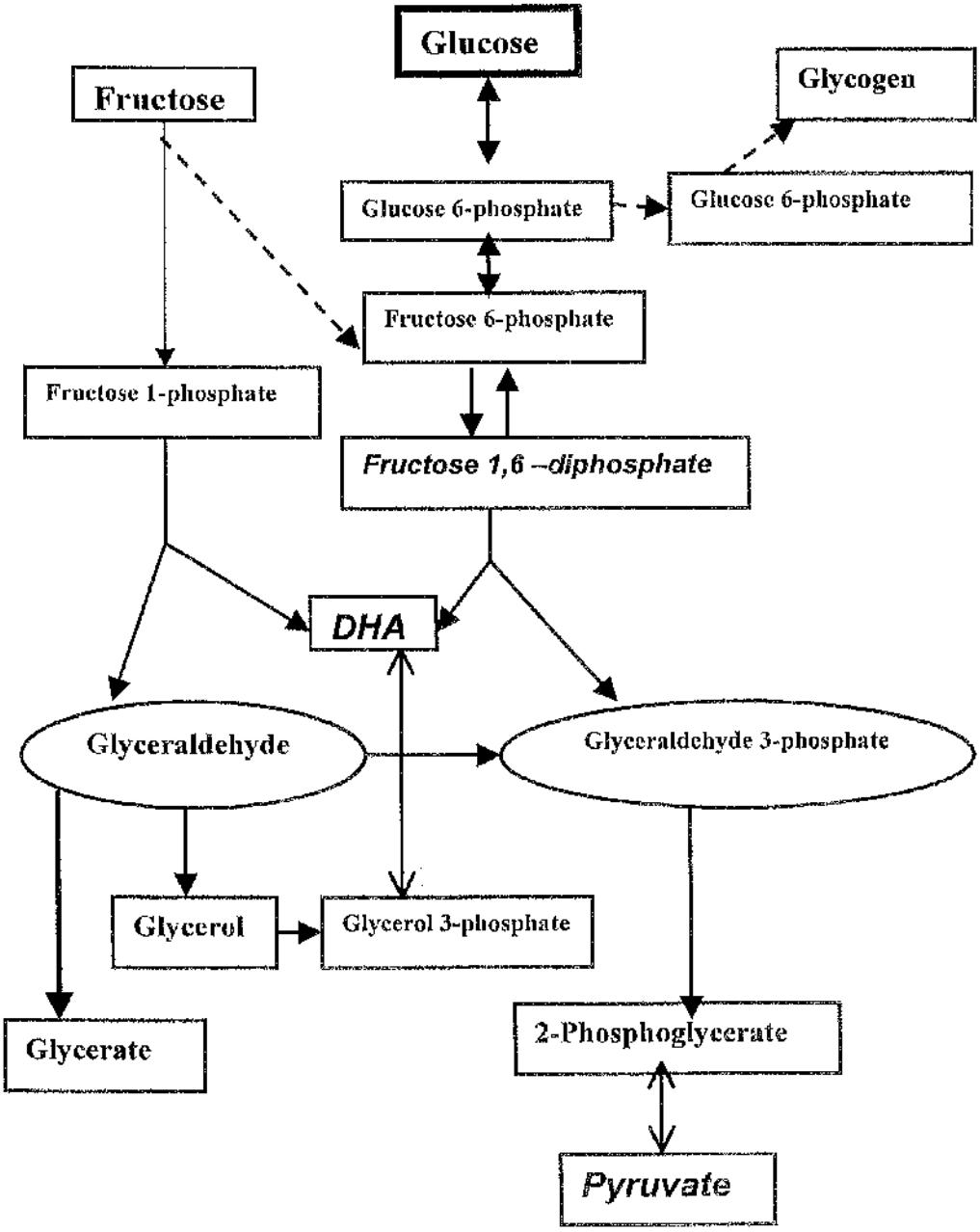
Glucose can be:

- Stored as glycogen (glycogenesis)
- Synthesised from non-carbohydrate sources (gluconeogenesis)
- Catabolised to provide energy (38 adenosine triphosphate (ATP) molecules/ glucose molecule)
- Converted to non-carbohydrate sources (e.g. non-essential amino acids)
- Converted to other carbohydrates or their derivatives (e.g. pentoses, uronic acids) or non-carbohydrate metabolites
- Converted to fatty acids.

The process for glucose catabolism occurs in the following phases:

- Glycolysis or Embden-Meyerhof pathway: the breakdown of glucose to pyruvic acid in the cytosol (Figure 1.1),
- Oxidation of pyruvate after transport into the mitochondria and acetyl CoA formation,
- Krebs or tricarboxilic acid (TCA) cycle: Oxidation of acetyl CoA from catabolism of carbohydrates, lipids and proteins into carbon dioxide, FADH2, NADH and GTP (Figure 1.2)
- Oxidative phosphorylation electron transport chain produces ATP and water (Frayn, 2003a).

Figure 1.1 Glycolysis (Embden-Meyerhof pathway)



DIIA: Dihydroxyacetone

DHAP: Dihydroxyacetone phosphate

Figure 1.2 Krebs cycle and the pathways that lead to it.

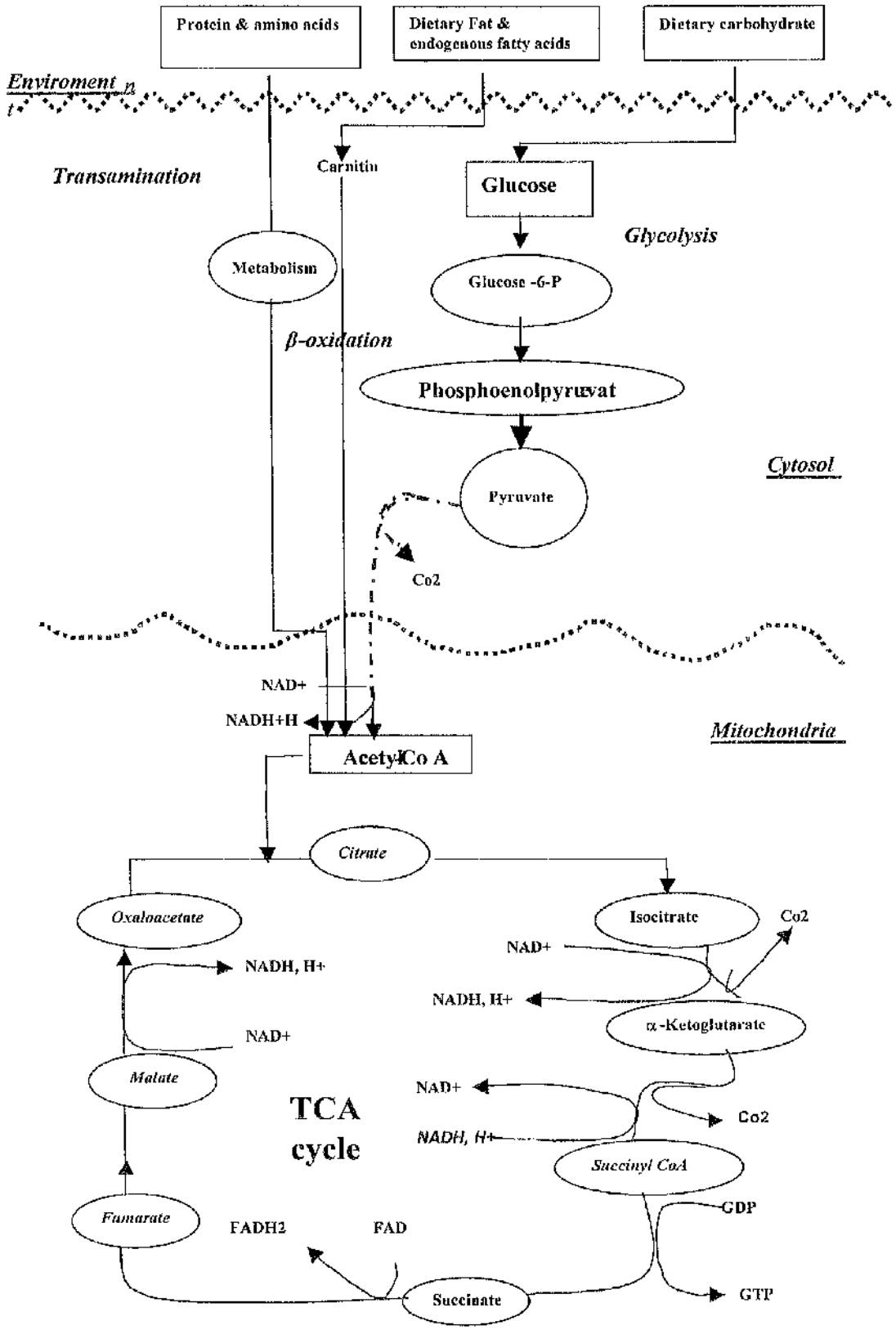


Figure 1.2 illustrates the main reactions leading to oxidation of various substrates in the TCA cycle. The electron transport system produces ATP, the main energy storage compound in living organisms from high-energy intermediates such as NADH, FADH2 and GTP produced during glycolysis and the TCA cycle. Its cleavage to ADP and inorganic phosphate fuels biochemical processes in cells. In this way, essential physiological processes such as muscle contraction, nerve action, and protein synthesis are possible. Thus oxidation of organic compounds in food to CO_2, accompanied with the formation of the reduced form of nicotinamide adenine dinucleotide and flavin adenine dinucleotide and (NAD and FADH2 coenzymes) provides the substrates for ATP synthetase linked to the enzyme complexes in the oxidative phosphorylation chain finally producing water. Glycolysis takes place in the cytosol, the TCA cycle takes place in the mitochondria and the electrons and hydrogen ions for ATP production are produced within the mitochondrian matrix (Rees and Howard, 1999). Important shunt pathways regulate the passage of intermediates between the cytoplasm and mitochondria regulating the process.

For each turn of the TCA cycle 12 ATP are produced for each citric acid, 15 ATP are produced from each pyruvic acid entry into the cycle. In this way, 38 ATP molecules are the result of the total oxidation of one glucose molecule. Any carbon entering the TCA cycle as Acetyl CoA leaves as CO_2 as indicated in figure 1.3. This means that fatty acids and some amino acids which enter as Acetyl CoA can not be used for gluconeogenesis which uses oxaloacetate as a precursor.

1.2.4 Physiological effects of carbohydrates

Carbohydrates are not only major sources of energy but also have various physiological effects. There are some aspects of carbohydrate structure that affect their physiological function, such as the rate of digestion, absorption and colonic fermentation (Table 1.3).

- The nature of the carbohydrate and absorbed monosaccarides is important in determining the effects on plasma lipids and provides a fundamental feature of this thesis. Glucose from digestible starch is absorbed entirely. However, only approximately half of the carbohydrate in fruit and dairy products provides glucose, the rest is composed of fructose or galactose which do not raise blood glucose or insulin levels (Lee and Wolever, 1998; Wolever, 2003).
- The amount of carbohydrate absorbed influences plasma glucose and insulin responses after oral consumption. Unabsorbed carbohydrates will enter the colon.
- The rate of carbohydrate absorption is an important factor in determining plasma glucose and in particular plasma insulin levels. Rate of absorption is affected by many factors such as food form, starch structure, particle size, food processing, viscosity, cooking temperature and moisture of the prepared meal as well as any factor which may delay gastric emptying such as fat or soluble fibre in the meal (Bjorck *et al.* 1994).
- The fermentation of carbohydrate by colonic bacteria results in the production of short-chain fatty acids (SCFA) such as acetate, propionate and butyrate (Jenkins *et al.* 1998). These may independently influence glucose and lipid metabolism. For instance, acetate has no direct effect on glucose

metabolism (Scheppach *et al.* 1988) however it results in reduction in plasma NEFA levels. Acetate is also incorporated into newly synthesised lipids. Wolever *et al.* in 1991 showed that rectal infusion of acetate (180 mmol), propionate (60 mmol) and propionate and acetate (180 mmol) increased blood lipids. Acetate increased serum cholesterol, glucagon, and acetate concentrations and reduced NEFA within 30 minutes. In contrast, propionate caused an increase in serum propionate, glucose, and glucagon with no effect on cholesterol level. The addition of propionate to acetate resulted in no significant increase in serum cholesterol. Therefore, colonic propionate is a gluconeogenic substrate in humans and inhibits the utilization of acetate for cholesterol synthesis (Wolever *et al.* 1991). SCFA may also have protective effects against chronic colonic diseases. A reduction of colon cancer risk was shown to be related to resistant starch intake (Kendall *et al.* 2004). Resistant starch enters the colon and increases the production of butyric acid. Butyric acid provides a fuel for the epithelial cells of the large intestine and promotes colonic health (Duncan *et al.* 2004). It has been shown that butyrate is a potential anti-cancer molecule as it stimulates apoptosis and inhibits histone deacetylase (Buda *et al.* 2003; Davie, 2003).

Table 1.3 Physiological effect of carbohydrate structure and digestibility

Physiological effect	Rapidly digestible carbohydrates	Non-digestible Carbohydrates
Energy	4 Kcal/g	2 Kcal/g if fermented
Gut motor activity	No effect	May delay gastric emptying and SBTT but speeds up colonic transit depends on viscosity
Satiety	No real effect	Could increase satiety if they slow gastric emptying
Blood glucose and Insulin	Increases rapidly	Slow increase or less insulin produced
Lipid metabolism and plasma lipids	Increase TAG concentrations Increase NEFA	If decrease acetate and increase propionate may decrease TAG levels and increase HDL-cholesterol levels
Colonic microflora	None	Increase fermentation Increase bacteria May have selected effects (prebiotic)
Bile acid Dehydroxylation	None	Increase
Protein glycation	Increases	No effect

SBTT: Small bowel transit time

1.2.5 Control of carbohydrate metabolism

Carbohydrate digestion and absorption is one of the highly efficient processes in the body. Only 5% of the energy intake is lost in faeces (Frayn, 2003a; Mendosa, 2005). The rate of glucose uptake is determined by the rate of hydrolysis of the carbohydrates, which are susceptible to pancreatic and intestinal enzymes. Factors

such as particle size, food structure, carbohydrate structure (e.g. ratio of amylose to amylopectin in starch), lipid content of food, presence of enzyme inhibitors, the rate of gastric emptying and transit time in the small intestine may influence this process, through the action of hormones such as cholecystokinin and gastrin and stimulation of duodenal and ileal receptors. For example, lipids in the ileum slow the transit of intestinal contents through the earlier parts of the small intestine (Jenkins *et al.* 2002c). Once absorbed the plasma levels of glucose are controlled by insulin and glucagon. Many effects of diet on plasma lipids are caused by changes in plasma levels or sensitivity to insulin and glucagon.

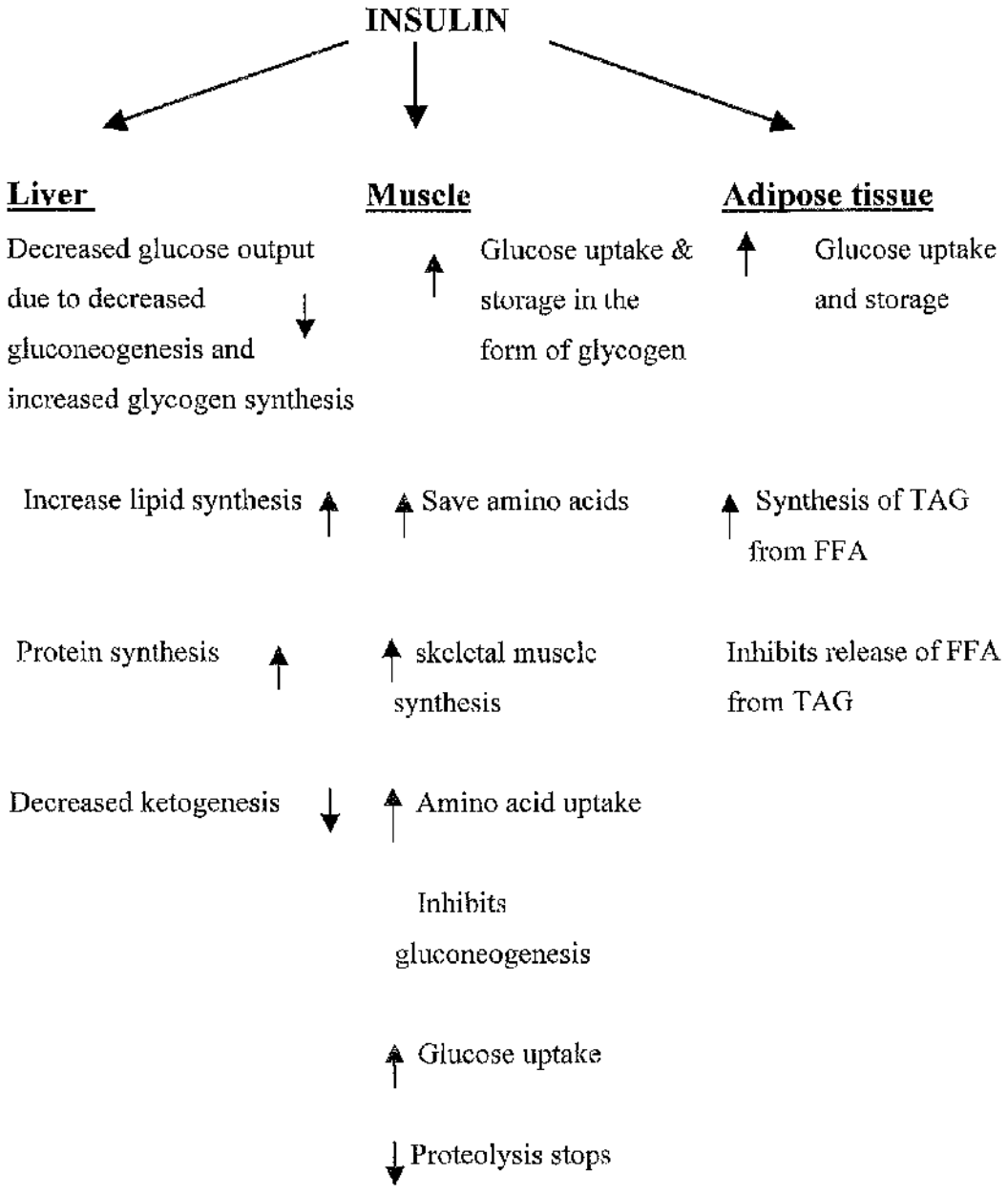
1.2.6 Insulin and Glucagon

1.2.6.1 Insulin

The β-cells of the pancreas produce a peptide hormone, called insulin, secreted in response to increased plasma glucose. Insulin has two types of action: stimulatory and inhibitory (Figure 1.3). Insulin stimulates glucose uptake by all tissues except the liver and the synthesis of new lipid and glycogen from glucose. It increases the uptake of glucose stimulating the movement of GLUT 4 transporters from the cytoplasm into the cell membrane. The liver does not have GLUT 4 and therefore is not affected. Instead insulin reduces the release of glucose by the liver. It also inhibits lipolysis, proteolysis, ketogenesis, glycolysis and gluconeogesis. When blood glucose is low and insulin levels decrease there is increased glycogenolysis and gluconeogensis in the liver, which in turn produces an increase in plasma glucose level. Low insulin also causes an increase in proteolysis in the muscle, which releases amino acids and after transamination, stimulates gluconeogenesis. Lipolysis in adipose tissues also increases and releases glycerol and free fatty acids

to act as fuels and for the glycerol to enter gluconeogenesis. Insulin therefore has an important role in maintaining blood glucose and releasing other substrate in a highly controlled manner for human metabolism (Sonksen and Sonksen, 2000; Pessin and Saltiel, 2000).

Figure 1.3 The actions of Insulin



1.2.6.2 Insulin Resistance

Insulin resistance is the resistance of the tissues to the physiological effects of insulin. It is thought to result from chronic high insulin levels, due perhaps to high intakes of fast release carbohydrate, low fibre diets and high adiposity. The high levels of insulin, in turn, down regulate the response of cells to normal levels of insulin. This can then result in the development of late onset or type II diabetes. The body has to produce more insulin to get the same action in response to normal postprandial glucose levels.

Apart from plasma glucose concentration, it has been shown that dietary macronutrient composition (e.g. carbohydrate, fat and protein) stimulates adipose tissue lipoprotein lipase (Yost *et al.* 1998) and insulin secretion (Daly, 2003). There is evidence that a high fat diet resulted in insulin resistance (Lovejoy *et al.* 1992) and higher risk of type II diabetes and CVD (Marshall *et al.* 1991). However, some studies failed to show any difference in insulin sensitivity between high fat (HF) and high carbohydrate (HC) diets (Swinburn *et al.*1991; Borkman *et al.* 1991).

Insulin resistance, which is the term given to the situation in which the actions of insulin are blunted in the presence of normal or increased insulin secretion (Gibney *et al.* 2005), is a cental feature in the development of type 2 diabetes and is now recognised to play a role in many of the risk factors for CHD such as abnormal lipid levels or dyslipidemia. It is also now recognised that insulin resistance may be the common link between obesity, impaired glucose tolerance or type 2 diabetes, dyslipidemia (high LDL cholesterol, low HDL cholesterol, high TAG

concentrations), hypertension and impaired fibrinolysis which together have been called metabolic syndrome or syndrome X (Reaven *et al.* 1996).

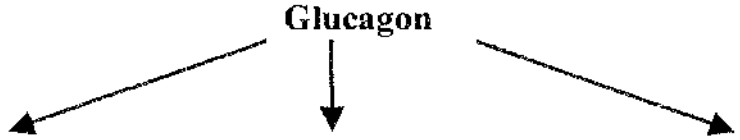
1.2.6.3 Glucagon

The action of insulin is balanced by the secretion of glucagon from the α-cells of the islets of Langerhans of the pancreas. Its action is almost the opposite of insulin. That is, it elevates blood glucose concentration by stimulating gluconeogenesis and inhibiting glycolysis in the liver. Glucagon does not affect glycogenolysis in muscle but stimulates glycogenolysis in the liver. Glucagon secretion is also regulated by amino acids. Glucagon opposes the action of insulin on lipogenesis by inhibiting the activity of acetyl-CoA carboxylase, which leads to an inhibition of lipogenesis.

Table 1.4 Factors affecting glucagon secretion (Jiang and Zhang, 2003)

Stimulators	**Inhibitors**
Glucogenic amino acids:	Glucose
Glycine, Alanin, Serin, Theronin &	Free fatty acids
Cystein	Ketones
CCK & Gastrin	Insulin
Cortisol	Secretin
Infections & other stresses	α-Adrenergic stimulors
Acetylcholine	
β-Adrenergic stimulators	

Figure 1.4 The actions of Glucagon.



Liver	Muscle	Adipose tissue
Glyconeogenesis is stimulated and the synthesised glucose is released,	No receptor, no effect for skeletal muscle, amino acid uptake and glycogen in skeletal muscle.	Stimulation of FFA release from TAG,

1.2.6.4 C-Peptide

Pancreatic β-cells secrete C-peptide which is the connecting part between the two subunits of insulin, in equimolar amounts to insulin (Rubenstein *et al.* 1969). It has an important physiological role in the biosynthesis of insulin and facilitates the formation of the disulphide bonds between the insulin subunits (Wahren, 2004). When proinsulin is converted to insulin in the pancreas, C-peptide is released. This peptide has been shown to increase glucose uptake into skeletal muscle cells without action through the insulin receptor (Zierth *et al.* 1996). The pancreatic secretion of insulin is well reflected by plasma C-peptide levels and high levels of C-peptide are associated with insulin resistance and development of chronic disease (Jenkins *et al.* 1988).

Results of C-peptide determination among 1999 healthy women from the Nurses' Health Study I and II showed that there was a significant and positive trend for a

relationship between C-peptide level and dietary glycaemic load. Similarly, subjects in the highest quintile of energy-adjusted, fructose intake had 13.9% higher C-peptide level than subjects in lowest quintile. C-peptide was negatively correlated with a higher intake of cereal (15.6% lower, *P* for trend=0.03) after control for other covariates.

An increased insulin level is associated with higher C-peptide levels and high intakes of fructose and high glycaemic index foods (Chen *et al.* 1999). Rapidly digestible carbohydrate leads to the development of resistance to the effects of insulin in normal diets. With higher C-peptide concentrations there will be higher metabolic disturbance,; whereas consumption of carbohydrates high in fibre, such as whole-grain foods, is associated with lower C-peptide and consequently insulin concentrations (Wu *et al.* 2004).

1.3 Dietary fats

Dietary fats are important organic components in the diet. Fats are composed of a carbon skeleton with hydrogen and oxygen substitutions and are the mose dense dietary source of energy (9kcal/g). Dietary fats also supply nutrients such as essential fatty racids (e.g. linoleic and linolenic acids) and fat-soluble vitamins (vitamins A, D, E and K). Although fats improve palatability of cooked food in the diet (Gibney, 1999), they significantly increase the risk of chronic and degenerative diseases and consequently, influence human morbidity and mortality (FAO/WHO, 2003).

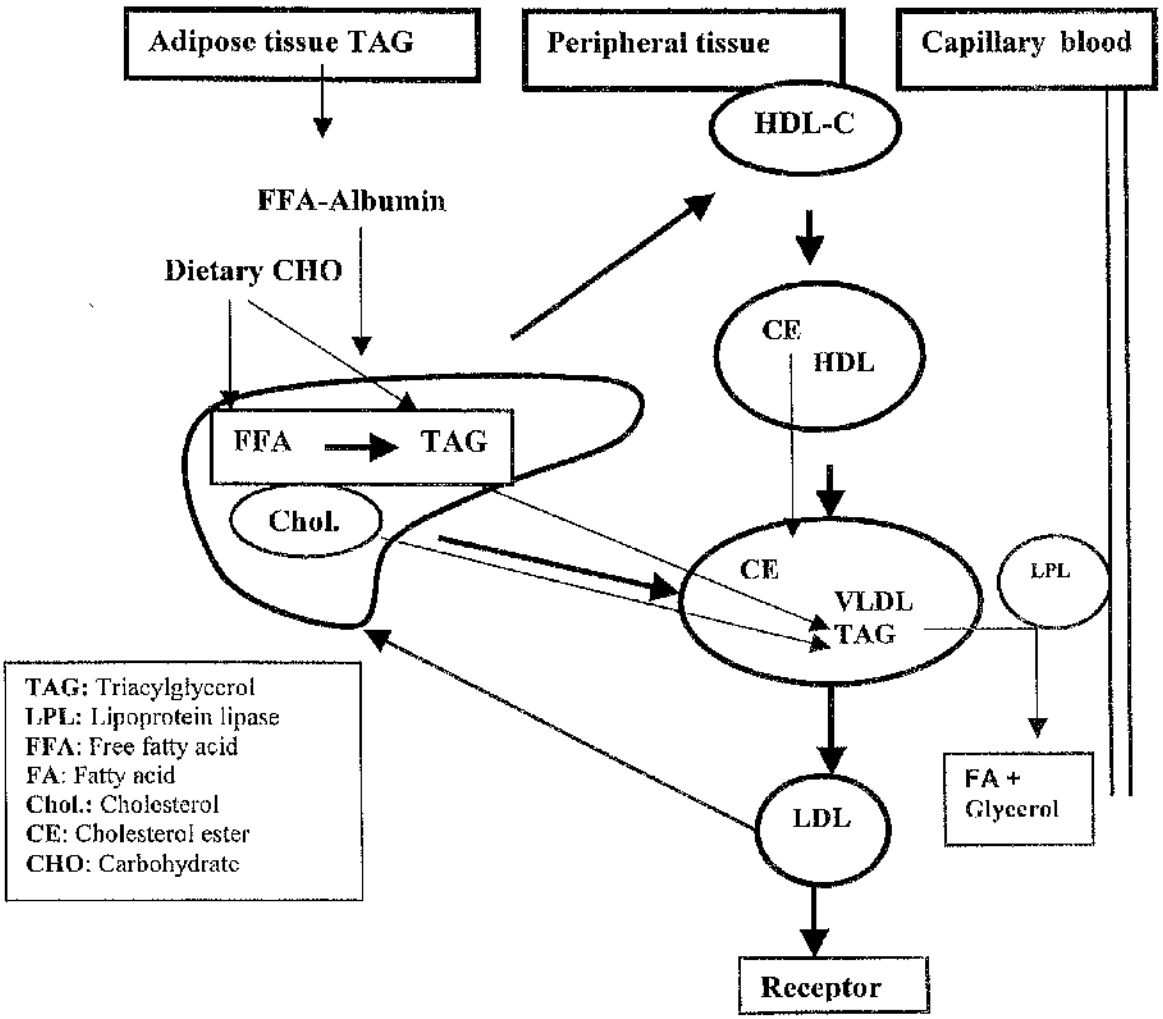
Lipids are classified into three categories based on their role in the body including provision of energy, structural (e.g. phosphoglycerides), storage (e.g. triacylglycerides) and metabolic lipids (e.g. steroid hormones). Fats are esters of fatty acids with glycerol. Therefore, the main components of dietaryfats are fatty acids, carboxylic acids with the structure of RCOOH. R is acyl carbon (varying in length from 4 to greater than 30, e.g. butyrate, stearate, palmitate). These fatty acids could be either saturated (e.g. palmitic and stearic acids), or monounsaturated (e.g. oleic acid) or polyunsaturated (e.g. α-linolenic acid) including one or more double bonds, respectively. Polyunsaturated fatty acids are further subclassified to n-3 (e.g. α-linolenic, docosahexanoic and eicosapentanoic acids) and n-6 fatty acids based on the position of first double bound in the fatty acid chain (close to CH3). n-3 fatty acids, mainly found in fish and marine products, have been shown to be associated with lower risk of myocardial infarction and CHD (Agostoni and Bruzzese, 1992). Sterols are another important group of lipids in human nutrition as a vital component in the membranes, precursors of bile salts for fat digestion and finally as precursors for steroid hormones (Frayn, 2003a).

The process of digestion and absorption of fats is shown in Table 1.2. Long chain fatty acids after absorption and esterification in the intestinal cells are converted to TAG and are packaged in the Golgi apparatus into chylomicrons. The chylomicrons are then secreted into lymphatic capillaries that surround the intestinal cells and via the larger lymph vessels to the thoracic duct, a one-way valve into the systemic circulation. Chylomicrons reach their highest level in circulating blood after a meal and as they pass around the circulation their TAG contents are hydrolysed by lipoprotein lipase mostly secreted by adipocytes and hepatocytes (Figure 1.4)

(Schaefer, 2002; Metzler, 2003). Most long-chain fatty acids are oxidised through the β-oxidation pathway.

Dietary fats from animal and plant sources consist of mainly TAGs and a small proportion of free fatty acids (FFA), particularly short-chain fatty acids (Department of Health, 1991).

Figure 1.5 Transport of lipids by plasma lipoproteins



1.3.1 Lipoproteins

Lipids, which are hydrophobic compounds, are the major stores of energy producing substances for human metabolism. These non-water soluble compounds are transported by lipoproteins in the circulating blood (Table 1.4). Lipoproteins are particles with a relatively hydrophilic surface and a highly hydrophobic core. Cholesterol esters, triglycerides (TAG), apoproteins, and phospholipids are components contained within each type of lipoprotein. Depending on the type of lipoprotein; the amount of each component varies. Major lipoproteins are classified into chylomicrons (CM), very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL) (Table 1.5; Olson, 1998; Fielding and Frayn, 1999).

Table 1.5 Lipoprotein classes

Major lipids in different lipoproteins	**Lipoprotein**	**Main composition (%)**	**Apolipoproteins**
Dietary TAG	Chylomicrons	TAG (90%)	B48, AI, AII, C and E
Endogenous TAG	VLDL	TAG (65%)	B100, C and E
Cholesterol and cholesterol ester	LDL	Cholesterol (45%)	B100
Cholesterol ester and phospholipids	HDL	Protein (50%)	AI, AII, C and E

(Olson, 1998; Frayn,, 2003a ; Redgrave, 2004 ; Manchekar *et al.* 2004)

Apoproteins are the protein constituents of lipoproteins. Apoproteins are categorised on the basis of their function into four classes A, B, C, and E. Apoprotein A is sub classified further to apo Al, All and AIII. Apo A-III was previously called Apo D (Connelly and Kuksis, 1981; Olson, 1998). The plasma lipid transport system includes various lipoproteins, their receptors at the cellular sites and different enzymes in the tissues and circulation involving in lipid metabolism (Goldberg and Schonfeld, 1985).

After lipid absorption, TAG is resynthesised and coated with phospholipids, apo A and apo B-48 in the enterocytes. The resulting large particles are chylomicrons (CMs,) which are the largest particles among lipoproteins. CMs are composed mainly of TAG (90%), proteins, cholesterol, cholesteryl esters and phospholipids (Olson, 1998). CMs are transported from the gut into the lymphatic system and finally via the thoracic duct into the bloodstream. When CMs enter into the tissues (e.g. adipose tissue), their TAG are cleaved and fatty acids are released by LPL action. The produced fatty acids are used by the tissues for energy or stored as TAG in adipose tissues (Redgrave, 2004). After LPL action, the CMs shrink into CM remnants. These remnants are recognised and taken up by apo E and apo B receptors in the liver. In this way, CMs are considered as the main dietary lipid carrier (Olson, 1998; Figures 1.6 and 1.7).

VLDLs transport endogenous produced TAG in the liver. Similar to CMs, TAG is the main constituent of VLDLs (65%). LPL hydrolyses the TAG inside VLDLs and release free fatty acids. The degradation of VLDLs results in VLDL remnants called

intermediate-density lipoprotein (IDL) which becomes LDL by further degradation (Hill and McQueen, 1997; Frayn, 2003b; Figures 1.6 and 1.7).

LDLs are the main carrier of cholesterol in the circulation. The LDL particles contain cholesterol and cholesteryl esters (65%) and protein (20%). The main apoprotein of LDLs is apo B-100. LDLs may be removed from the circulation by either LDL receptors or scavenger routes. These routes seem to be important when the LDL levels are high and cholesterol is incorporated into atheromatous plaques (Hill and McQueen, 1997; Frayn, 2003a).

HDLs are the smallest lipoproteins. The high proportion of protein in HDL causes its high density. The main apoproteins of HDL are apo AI and apo AII. HDL particles are derived from gut and also the liver. HDLs originated from the liver are HDL3, which are discoid shaped lipoproteins. By transferring free cholesterol and phospholipids released by LPL from TAG-rich lipoproteins (chylomicrons and VLDL) to HDL3 via LCAT action, HDL2 (a spherical shaped HDL) is produced (Figure 1.5). HDL2 contains 50% protein and 20% cholesterol and cholesteryl esters. HDL2 takes free cholesterol to the liver and is converted to pre-β HDL or apo AI which are ready for further circulation (Olson, 1998; Frayn, 2003a; Redgrave, 2004; Manchekar *et al.* 2004). When plasma TAG increases, HDL transfers cholesteryl esters into TAG-rich lipoprotein via cholesteryl ester transferase protein (CETP). Thus HDL transfers cholesterol to the liver and reduces of plaque formation and CHD.

The different classes of lipoproteins interact with each other and exchange contents. Two interconnected exogenous and endogenous cycles in lipoprotein metabolism exist and the liver plays pivotal role in their metabolism (Hill and McQueen, 1997). Two enzymes, lipoprotein lipase (LPL) and lecithin:cholesterol acyl transferase (LCAT) are key elements in lipoprotein metabolism. LPL in the tissues and mainly adipose tissue releases free fatty acids and glycerol from chylomicrons and VLDL into the tissues while LCAT forms cholesterol esters from free cholesterol and fatty acids (Hill and Mc Queen, 1997). There are four main steps in the reverse cholesterol transport (RCT) system exist (ie movement of cholesterol to the liver). Binding of the free cholesterol from peripheral tissues by HDL particles causes an efflux of free cholesterol. The second step is the conversion of free cholesterol in HDL particles to cholesterol esters by LCAT. The transfer of cholesteryl ester from HDL to apo B containing particles (VLDL and LDL) in exchange for TAG is the third step which is facilitated by cholesterol transfer protein (CETP). Subsequently, the produced cholesterol is taken up by the liver through the specific receptors for LDL or returned to the periphery (Hill and McQueen, 1997).

Transfer of lipids between the plasma lipoproteins is catalysed by CETP and this protein clears cholesterol from peripheral tissues (Bruce *et al.* 1998). CETP has a two-fold action in cholesterol metabolism. One is transferring cholesterol from HDL to VLDL and LDL. A reduction in activity of CETP level leads to an increase in HDL level (Hill and McQueen, 1997). However, other studies have shown that CETP action results in the production of pre-β-1 HDL in the degradation of HDL2 and therefore may lead to an increase in HDL levels similar to the protective effects of

moderate alcohol consumption (Hannuksela *et al.* 1992). Therefore, HDLs act as a cholesteryl ester shuttle (Figure 1.5 and Figure 1.7).

Figure 1.6 shows the relationships between chylomicrons from the gut and VLDL from the liver. Dietary lipids and *in vivo* biosynthesised lipids enter the metabolic system through the liver and peripheral tissues (Goldberg and Schonfeld, 1985; Olson, 1998)

Figure 1.6 HDL and lipid metabolism (Olson, 1998; Frayn, 2003a; Redgrave, 2004; Manchekar *et al.* 2004)

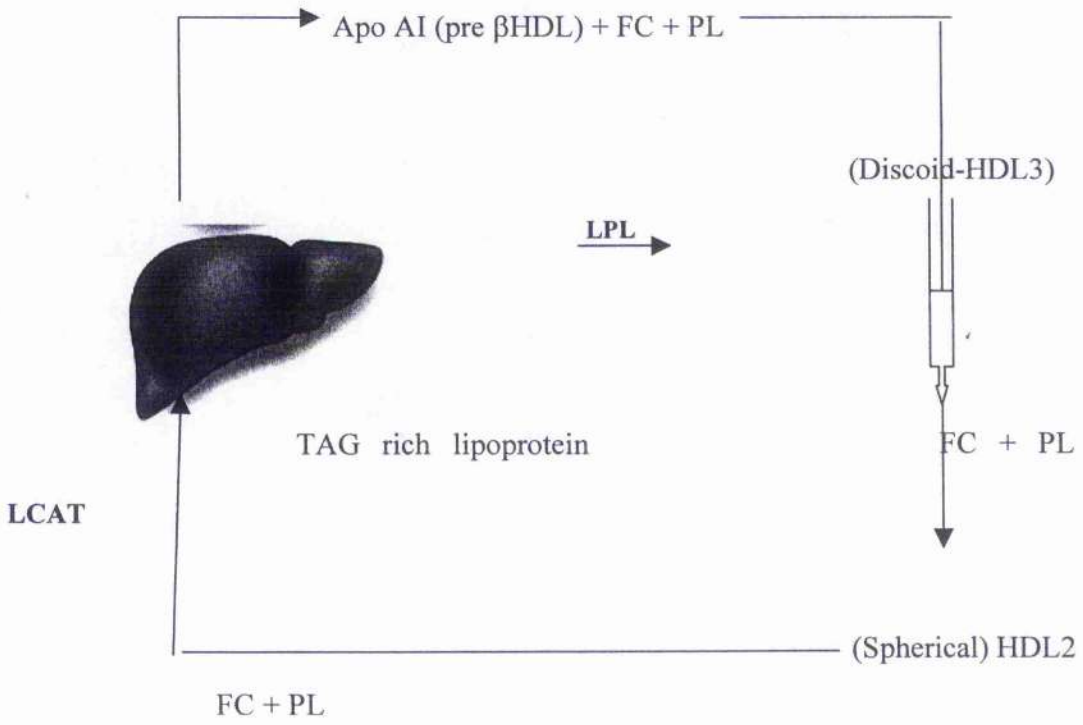


Figure 1.7 Comparison of the fate of dietary lipid and newly synthesised lipid as it enters the circulation

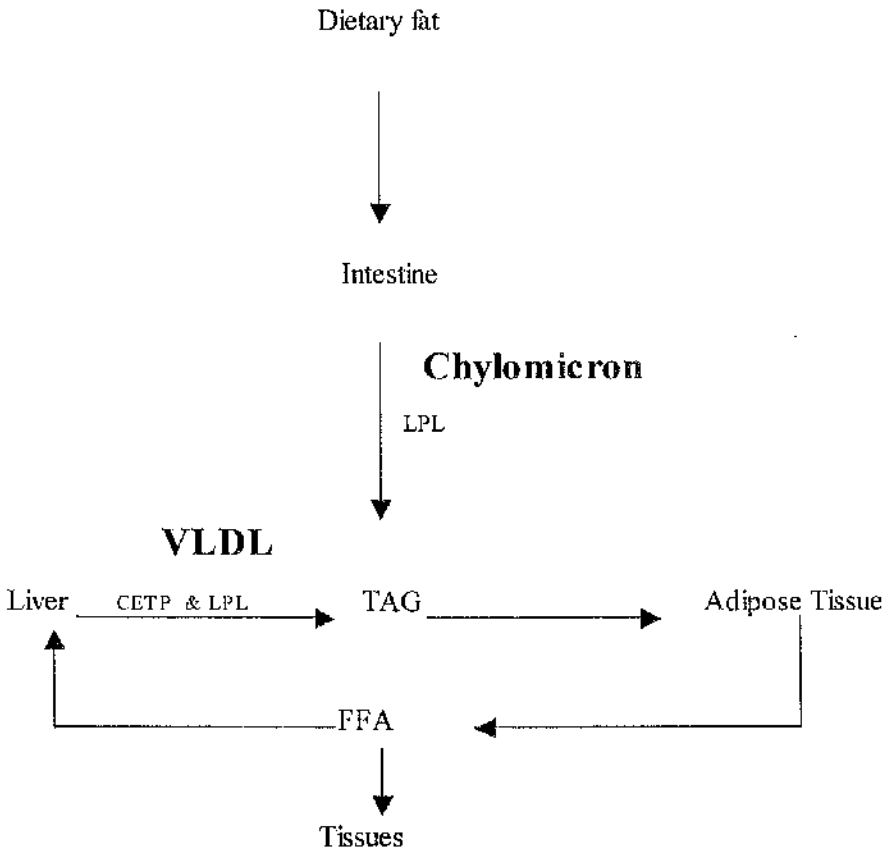
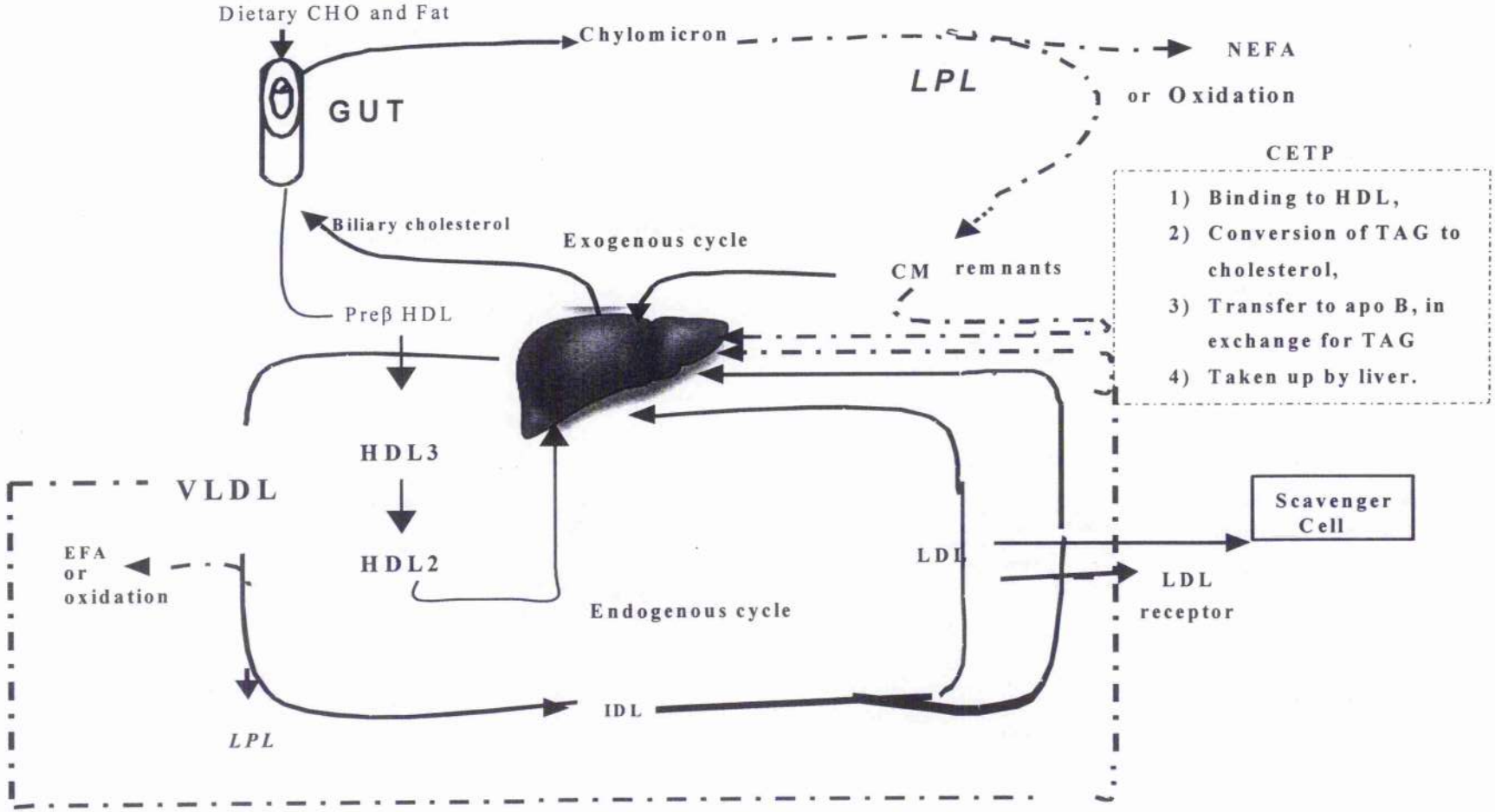


Figure 1.8 Overview of exogenous and endogenous lipoprotein metabolism



1.4 Interrelationship between metabolism of dietary carbohydrate and fat

There are many interactions between the metabolism of carbohydrates and fats (Randle, 1998; Fried and Rao, 2003; Frayn, 2003b). Carbohydrates provide the carbon skeleton for fatty acid synthesis. Oxidation of carbohydrate produces acetyl residues that are exported from the mitochondria as citrate and then converted to fatty acyl-CoA. Acyl-CoA is then incorporated into TAG which is mainly exported from the liver as VLDL (Fried and Rao, 2003; Frayn, 2003b). TAG is synthesised every day by the liver (≈40-100g/day) from dietary carbohydrates and free fatty acids. If the amount of consumed carbohydrates exceeds requirements, carbohydrates are converted to TAG within hepatocytes and adipocytes (Austin, 1997; Fried and Rao, 2003) and hepatic VLDL secretion is increased. In this way high-carbohydrate diets may act in a similar way to high-fat diets resulting in higher levels of plasma TAG (Truswell, 1994).

Excess carbohydrate leads to an increase in movement of glucose and fructose through glycolysis. This increases acetyl CoA that then provides more substrates for *De novo* fatty acid synthesis (Frayn and Kingman, 1995).

Carbohydrate feeding through the action of insulin also up-regulates the activity of the enzymes, which synthesize fatty acids. The activity of hepatic synthases and the NADPH-generating enzymes significantly increases in rats fed on glucose and fructose, which in turn led to a significant increase in the rate of TAG secretion (Kazumi *et al.* 1997).

It has been shown that a low-fat diet in healthy subjects caused a reduction in plasma LDL-cholesterol concentration (Dreon *et al.* 1997). In another study, consumption of a low-fat diet resulted in a change in LDL pattern in 44% of subjects (Krauss and Dreon, 1995). The low-fat diet also significantly increased plasma TAG concentrations and reduced HDL-cholesterol concentrations (Parks *et al.* 1999).Thus consumption of high carbohydrate diet could induce fatty acid biosynthesis through *De novo* lipogenesis by carbohydrate over feeding of either glucose or fructose.

1.4.1 Insulin and hepatic TAG secretion

TAG is stored in adipose tissue (adipocytes), and is produced by intracellular lipoprotein lipase (LPL). After that hormone sensitive lipase (HSL) action leads to the production of NEFA (Frayn, 1998). Insulin and NEFA levels are related, as when insulin level increases after meal, plasma NEFA is suppressed (Singer *et al.* 1985). Insulin reduces the hydrolysis of lipids in adipose tissues and causes an inhibition in VLDL-TAG secretion. In this way, resistance to the effects of insulin could result in a chronic increase in circulating NEFA levels by increasing the rate of lipolysis in the liver. In addition, some resistance to the physiological effects of insulin can develop in less physically active people (Zammit *et al.* 2001). The activity of muscles, which are the major site for insulin dependent glucose metabolism, and also the type of diet and its constituents contribute to the development of this disorder which is a critical point in the development of insulin resistance.

High fructose intake (Taghibiglou *et al.* 2000) or consumption of high fat diets (Boden *et al.* 1995) in human subjects or in experimental animals blocks the enzyme that catalyzes long-chain fatty acids β-oxidation within mitochondria (Carnitine

palmitoyl transferase I), which produces resistance to the effects of insulin. If the oxidation of fatty acids exceeds their metabolism, they divert toward glyceride synthesis.

Increased FFA and glucose levels that regulate VLDL output from the liver and elevate TAG concentrations inhibit apo-B degradation also caused by an increase in VLDL secretion. Meanwhile, lipoprotein lipase levels decrease and result in decreased clearance of VLDL and the final result would be more TAG rich particles, fewer HDL and smaller LDL particles leading to insulin resistance. Impaired VLDL lipolysis which depletes HDL by delaying the transfer of apoproteins from TAG rich lipoproteins to the HDL and increased hepatic lipase activity to facilitate HDL clearance are some of mechanisms of the decreased levels of HDL in the mentioned conditions (Howard, 1999).

Decreased HDL-C concentration and reduction in LDL particles size, which is equal to an increase in concentration of circulating small dense LDL lipoproteins, are the metabolic consequences that develop in response to an increase in TAG levels. They are the major components of dyslipidemia which occur in insulin resistance (Howard, 1999).

1.4.2 Glucose- fatty acid cycle

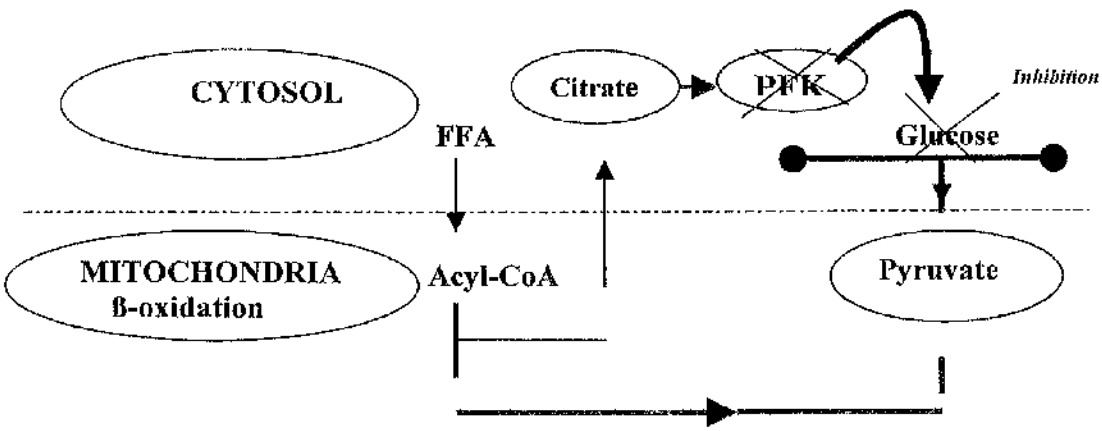
Randel introduced the concept of a Glucose-Fatty acid cycle in 1963. It integrates fatty acid and glucose metabolism (Randle, 1963), as if FFA could produce inhibitory effect for glucose metabolism as an oxidative fuel. The presence of FFA determines the rate of fat oxidation and directly inhibits glucose metabolism (Guerre-

Millo, 2003; Frayn, 2003b). A series of studies have confirmed a mechanism in which an increase in fatty acid oxidation reduces glucose uptake and oxidation in muscle tissues and the major role of this mechanism in reducing insulin sensitivity and glucose utilisation that can lead to insulin resistance (Belfiore *et al.* 1998).

High FFA in the cell promotes fatty acid oxidation while it inhibits glucose oxidation (Beshel' *et al.* 2003). This phenomenon also promotes glycogen synthesis accompanied by more fat storage (Randle, 1998). A central point in this relationship is that fatty acids reduce glucose oxidation and uptake in muscle tissues (Frayn ,2003b). Fatty acid oxidation can lead to reduced glucose utilisation. High levels of fatty acid have been shown to stimulate glucose production similar to what happens in type 2 diabetes which is followed by stimulation of glucose production and deterioration of glucose intolerance (Frayn, 2003b). An increase in actyl-CoA production by more β-oxidation of FFA is an important issue. Acetyl-CoA as an inhibitor of pyruvate dehydrogenase, is required for oxidative utilisation of glucose (Randle, 1998; Hegarty *et al.* 2003).

The glucose-fatty acid cycle and the probable effects of increased availability of acetyl-CoA and β-oxidation of FFA are summarised in Figure 1.9. The high ratio of fatty acid oxidation and the resultant acetyl-CoA develop high concentration of citrate through citrate synthase. In addition, the ratios of $NADH/NAD^+$ and ATP/ADP increase. High acetyl-CoA/CoA and $NADH/NAD^+$ ratios inhibit pyruvate dehydrogenase and in this way oxidation of pyruvate from glycolysis is suppressed.

Figure 1.9 Glucose-fatty acid cycle

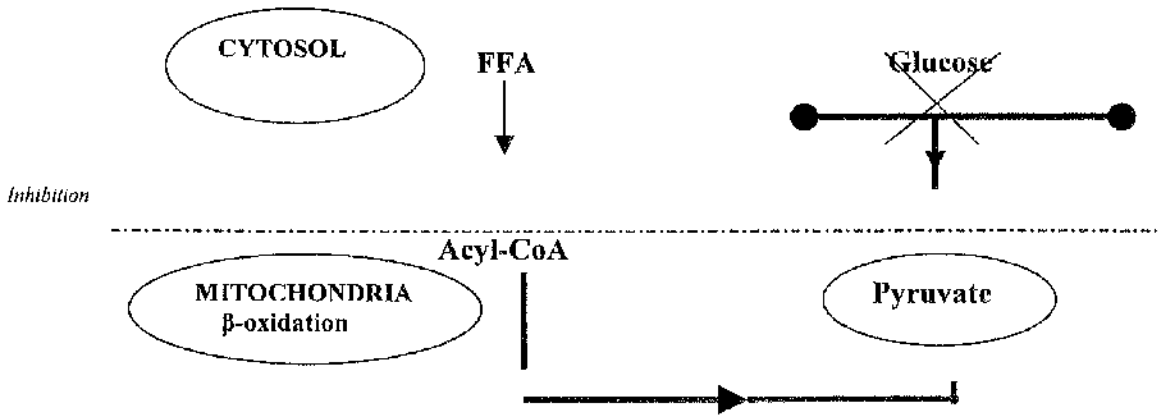


Three mechanisms have been hypothesised to explain the inhibitory effects of fatty acids on glucose oxidation through this cycle:

- The inhibition of pyruvate dehydrogenase which mediated by increased ratio of acetyl-CoA to CoA,
- The inhibition of phosphofructokinase by an increase in citrate,
- The inhibition of hexokinase by glucose-6-phosphate (Randle *et al.* 1994).

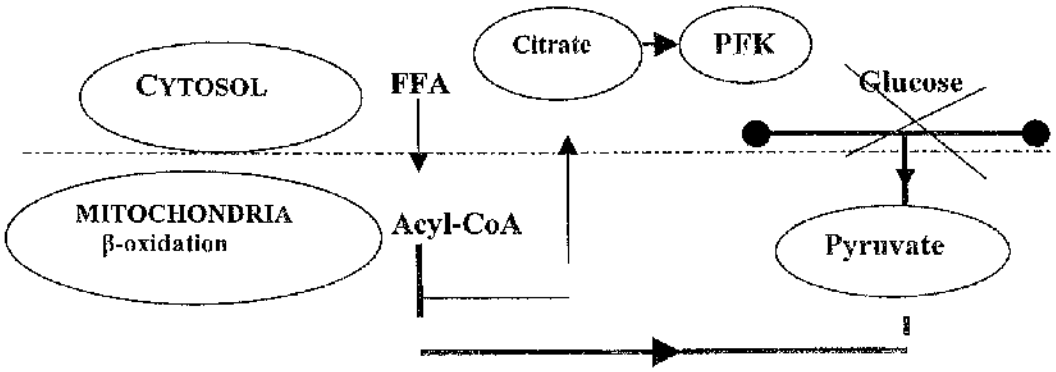
Figure 1.10 shows the inhibitory effects of fatty acids on glucose metabolism.

Figure 1.10 Inhibitory effect of FFA on glucose metabolism



Formation of citrate from acetyl-CoA can inhibit another glycolytic enzyme, phosphofructokinase, which diminishes glucose utilisation (Figure 1.11; Belfiore *et al.* 1998).

Figure 1.11 Inhibitory effect of citrate on glucose oxidation



The main mechanism for the glucose-fatty acid cycle is when fatty acid oxidation in muscle reduces glucose uptake and oxidation. The link between lipid accumulation and insulin resistance goes beyond the classic glucose-fatty acid cycle. A mechanism

for insulin resistance and its major role in reducing insulin sensitivity and glucose utilisation has recently been proposed. It is postulated that accumulation of long-chain fatty acyl-CoA plays a critical role and leads to insulin resistance (Hegarty *et al.* 2003).

Thus, the glucose-fatty acid cycle is not a metabolic cycle and does not show the interconversion of glucose-fatty acid, but it represents a coordination of a series of metabolic regulations in glucose and fat metabolism. When the concentrations of insulin and glucose are high, the malonyl CoA produced suppresses fatty acid oxidation, however, when the oxidation of fatty acids increase and the produced citrate inhibits pyruvate dehydrogenase, glucose uptake and oxidation reduce. Simply elevated glucose levels cause stimulation in insulin production and suppresses FFA release from fat depots (Frayn, 2003b).

The fatty acids released by the physiologic action of LPL may be isolated in adipose tissue. The released fatty acids may be esterified or released as Non-Esterified Fatty Acids (NEFA) into circulating blood. NEFA or free fatty acids are metabolic fuels and account for a greater variation in energy flux compared to glucose and reflect nutritional status and physical activity (Frayn *et al.* 1997). Acute elevation of NEFA causes hyperinsulinemia without an effect on insulin secretion rate in healthy subjects. This condition has been observed in obesity; impaired glucose tolerance, diabetes and dislipidemia linked to insulin resistance and hyperinsulinemia (Balent *et al.* 2002) and similar metabolic disorders.

1.5 Carbohydrate and fat requirements and recommendations

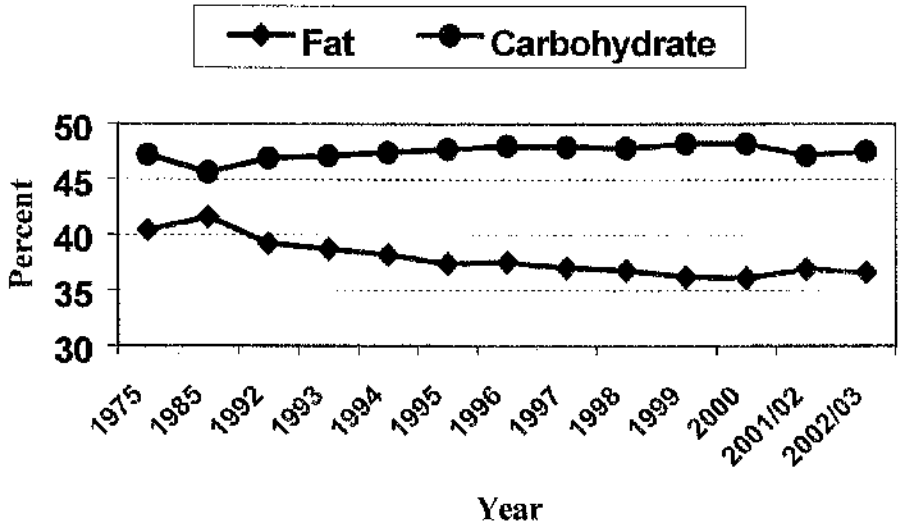
Carbohydrate is an important nutrient for different organs in the body, particularly for the neural tissues. Unlike other tissues, neural tissue preferentially uses glucose as fuel and adapts to oxidation of ketone bodies only after prolonged fast (Wolever, 2003).

The minimum amount of glucose needed for brain utilisation is 130 g/day. Therefore, carbohydrate should be at least 25% of a 2000 kcal diet (Jenkins *et al.* 2004). In very low carbohydrate diets (e.g. Atkins diet with <20 g/day carbohydrate content), fat and protein must supply the rest of required energy, this may adversely affect health because of ketosis and also because vegetables and fruits may be restricted. This type of diet may also not meet other nutrient requirements including vitamins and minerals.

Results of per capita energy and nutrient intakes from household food and drink in the UK have shown a longterm decrease in energy intake between 1975 and 2002-03 (from 2489 kcal/day to 2091 kcal/day, respectively). Average fat intakes have also decreased steadily over the period (≈24%), particularly by decreasing saturated and monounsaturated fatty acids. Regarding carbohydrate intakes, there was a reduction between 1975 and 1991 (331 g/person/day compared with 250 g/person/day). Per capita intake of carbohydrates was around 270 g/day from 1992 to 2000 and then it has been reduced by 6.9% (average intake was 265 g/day in 2002-03). In term of percentage of energy, there has been no significant change in carbohydrate intakes (47.2% in 1975 and 47.5% in 2002-03; Figure 1.12). Non-milk extrinsic sugars which are usually referred as added sugars has decreased from 87 g/person/day to 82

g/person/day while no significant changes were found for total sugar and starch intakes since 1975 (Department of Agriculture, 2004).

Figure 1.12 Contribution of fat and carbohydrate in energy intake (Department of Agriculture, 2004)



Recommended daily amounts (RDAs) for energy and nutrients in the UK were set in 1979. In 1987, the Committee on Medical Aspects of Food Policy (COMA) reviewed the RDA and Dietary Reference Value (DRV) was set (Committee on Medical Aspects of Food Policy 1984).

The definition of DRV for nutrients is usually based on estimation of average requirements for different groups of the population. The basic assumption in the DRV definition is that nutrient requirements are normally distributed. There are also some limitations for a number of nutrients and no DRVs are available. For example, as dietary carbohydrate and fat consist of variety of groups with different chemical and physiological properties, no exact DRV could be defined or estimated for fat,

fatty acids, starches and sugars. However, some dietary guidelines have been proposed such as contribution of starch or sugars to the energy intake (Department of Health, 1991). Recommendations for carbohydrate and fat intake as DRV are given as the desirable contribution of these macronutrients to either daily total energy intake (including alcohol) or food energy. The acceptable carbohydrate intake ranges from 45% to 65% of daily energy intake (Jenkins *et al.* 2004). For example, the recommended DRV for carbohydrate and fat are 50% and 35% of food energy in the UK, respectively (Table 1.6, Department of Health, 1991). The average intake of fat and carbohydrates in the British adults is summarised in Table 1.7.

Table 1.6 DRV for fat and carbohydrates for adults as a percentage of daily energy intake

	Population average (%)	
	Total energy	**Food energy**
Total fat	33	35
Saturated fatty acids	10	11
Cis-polyunsaturated fatty acids	6	6.5
Cis-monounsaturated fatty acids	12	13
Trans-fatty acids	2	2
Total fatty acids	30	32.5
Total carbohydrates	47	50
Non-milk extrinsic sugars	10	11
Intrinsic and milk sugars and starch	37	39
Non-starch polysaccharides (g/day)	18	N/A

(Department of Health, 1991).

Table 1.7 Daily intakes of energy, fat and carbohydrates by the British population

	Men (N=1087)	**Women (N=1110)**	**Total**
Energy (kcal)	2450	1680	2061
Total fat (g)	102.3	73.5	87.8
Carbohydrates (g)	272	193	232
Sugars (g)	115	86	100
Starch (g)	156	106	130
Non-starch polysaccharides (g)	11.2	12.5	11.6

(Department of Health 1991)

1.5.1 Simple sugars

Highly refined carbohydrates could be considered as 'empty calories' as they provide energy but not any essential nutrients (Department of Health, 1991; Jenkins *et al.* 2004). Although carbohydrate intake has not shown a marked change, simple sugar consumption has increased. For instance, in the United States, dietary carbohydrates showed a steady decrease from 500g/day (in 1963) to 374g/day (in 1991) and then it increased back to 500 g/day after 34 years (in 1997). The increase in dietary carbohydrate was parallel to an increase in not only highly refined carbohydrates but also increases in total fat and energy and a decrease in dietary fibre intake (Gross *et al.* 2004). Refined sugars are easily digestible carbohydrates A number of studies have shown that these type of carbohydrates are associated with higher risk or incidence of chronic diseases such as obesity, type 2 diabetes, insulin resistance and CHD (Katan *et al.* 1997; Parks 2001; Fried and Rao 2003; Jenkins *et al.* 2004).

Simple sugars in the diet are derived from those, which occur naturally in foods (e.g. lactose in milk, fructose in fruits) called intrinsic sugars and those, which are added

to the foods during food processing and production, called non-milk extrinsic sugars (NMES). There are two different recommendations:

(1) no limitation has been recommended for intrinsic or milk sugars,

(2) intake of NMES should not exceed 10% of total energy intake (Department of Health, 1991).

1.5.2 Fructose and high fructose corn syrup

Dietary carbohydrate intake increased since 1963 by 126 g per day in the USA and the average daily intake of fructose is from 19g per day to 37g (Glinsmann and Parks, 1995). Consequently, consumption of fructose has increased, because soft drinks and some foods have been sweetened by manufacturers with corn syrup. Although it does not stimulate insulin secretion from β-cells and in the postprandial state and it has a smaller postprandial insulin secretion effect than glucose containing foods and beverages (Elliott *et al.* 2002), fructose causes a dramatic increase in circulating TAG level. There was an exaggerated postprandial lipemia after fructose feeding revealed by a significant increase at TAG level (Jeppessen *et al.* 1995). They showed that adding fructose (50g) to the standard fat load for 11 healthy adult subjects meals, resulted in a higher postprandial TAG level. In this way, TAG-rich lipoproteins of intestinal origin may play a role in the fructose-induced increase of postprandial lipemia. The other suggested mechanism for this observation would be an impaired TAG clearance.

Although the reason for the different effects of fructose on TAG level is not yet clear (Jeppessen *et al.* 1995; Grant *et al.* 1994; Arefaine *et al.* 1998), this monosaccharide has less glycaemic effect and induces lower insulin secretion than glucose and starch.

Pancreatic β cells are not stimulated by fructose intake, so foods and beverages, which contain fructose, have smaller effects on postprandial insulin level than glucose (Elliott *et al.* 2002). However, the increase in the prevalence of obesity, which is attributed to increase in dietary intake of fructose containing food and beverages is linked to insulin resistance (American Diabetes Association, 2000).

The increase in carbohydrate intake in developed countries is associated with increased intake in fructose containing corn syrup and this has expanded dramatically, because soft drinks and some foods have been sweetened by manufacturers to produce more tasty foods (Gross *et al.* 2004; Bray *et al.* 2004). Although dietary fructose reduces circulating insulin level it increases TAG levels and consequently worsens the lipid profile (Jenkins *et al.* 2002c; Wu *et al.* 2003; Teff and Townsend 2004; Elliot *et al.* 2002).

1.5.3 Starch

Starches are the major type of dietary polysaccharides. Starchy foods not only play an important role in energy provision for the body but also contain other nutrients. Starches are classified according to the rate of digestibility due to physical state of starch granules such as rapidly digestible starch (e.g. found in bread, cooked rice and potatoes), slowly digestible starch (e.g. raw cereals, beans and whole grains) and resistant starch (e.g. raw potatoes). Resistant starch, which escapes digestion in the small intestine, is a substrate for colonic fermentation and results in production of short-chain fatty acids. These fatty acids are absorbed and contribute up to 10% of body energy supply (Department of Health, 1991; Jenkins *et al.* 1998). The ratio of simple to complex carbohydrate (i.e. sugar/starch ratio) in the diet may influence

fatty acid synthesis. That is lower (40:60) compared with the higher (60:40) sugar to starch ratio in a low-fat food resulted in trace increase in *de novo* fatty acids in VLDL-TAG (Hudgins *et al.* 1996).

Starch accounts for around half of the carbohydrate intake by the British population. The DRV for starch accompanied by intrinsic and milk sugars is that it should provide 39% of daily food energy (Department of Health, 1991).

1.5.4 Dietary fibre and non-starch polysaccharides

One of the major factors influencing digestability and the rate of absorption of carbohydrate foods, and hence postprandial glycaemia, is their dietary fibre content. In 1976 Trowell defined dietary fibre as the edible parts of plants and the remnants of plant cells that are resistant to hydrolysis by human enzymes. This includes all indigestible polysaccharides that undergo full or partial fermentation in the large intestine (Trowell *et al.* 1976) and include cellulose, hemicelluloses, lignin, pentrosans, pectins, gums, waxes, mucilages, modified cellulose and some processed polysaccharides. Dietary fibres are not nutrients, but they produce important physiological effects on human metabolism. (Table 1.8)

The term dietary fibre in the UK was later replaced by non-starch polysaccharides (NSP), which include cellulose and non-cellulosic polysaccharides (British Nutrition Foundation Task Force, 1990 and Prosky, 2000a). These are compounds in the plant cell walls such as pectin, cellulose, hemicellulose, pentosan and gums (e.g. guar gum and arabic gum). Fruits, vegetables, legumes, and cereals are NSP rich foods. NSP are categorised into soluble and insoluble NSP. Soluble NSP may form viscous gels

in the small intestine, which can reduce the absorption of other nutrients such as carbohydrate and fat (Edwards and Parrett, 1994). They are fermented by the colonic bacteria and produce SCFA (acetate, propionate and butyrate in the approximate molar ratio of 60: 25: 15) and gas. Insoluble NSP have little effect in the small intestine and are relatively resistant to bacterial fermentation and maintain their water holding capacity (WHC), which increases stool output (Cummings and Englyst, 1987). Cellulose and lignin are examples of insoluble NSP. The ratio of soluble to insoluble (S/I ratio) NSP in food varies. For instance, fruits and vegetables have higher S/I ratio whereas wheat has a lower ratio.

The physiological effects of NSP are summarised below in Table 1.8.

Table 1.8 Physiological effects of NSP

Function	Soluble NSP	Insoluble NSP
Gel formation	Increase[a]	-
Water Holding Capacity	-	Increase[a]
Colonic fermentation	Increase[b]	Slow or resistant[b]
Gas production	Increase[e&b]	-
Transit time	Decrease	Decrease[a]
Water absorption in the colon	Increase[b]	-
Stool output	-	Increase[a,b]
Absorption in GI	Fermentation[c]	-
Gastric emptying	-	Increase[a,b]
Blood cholesterol	Decrease[f]	Decrease due to increase in bile excretion[f]
Blood glucose	Decrease[c &]	-
Insulin sensitivity	Increased[c&d]	Increase[c& d]

([a]Cummings and Englyst, 1987;[b]Prosky, 2000a;[c]Jenkins et al, 1978; [d]Uchenna, *et al.* 1998; [e]Arjmandi *et al.* 1992a; [f]Turner *et al.* 1990).

The current recommendations are to encourage people to consume more NSP rich foods because of their beneficial effects. Consumption of NSP have been shown to be associated with an increase in insulin sensitivity, anti-carcinogenic effects in large bowel, decreased risk for type 2 diabetes (Salmeron *et al.* 1997a; Salmeron *et al.* 1997b; Uchenna *et al.* 1998; Gross *et al.* 2004), and CVD (Jenkins *et al.* 2002) (Department of Health, 1991; Bessesen, 2001b; Brand-Miller *et al.* 2003). The average intake of NSP in the UK is between 11 to 13 g/day, of which around half and 40% is provided by vegetables, and cereals, respectively. The DRV for NSP is an average 18 g/day (ranged from 12-24 g/day) from a variety of foods including fruits, vegetables, cereals (Department of Health, 1991).

1.5.5 Dietary Fat

Obesity and especially abdominal obesity is an important metabolic risk factor for insulin resistance, which is increasing worldwide. The prevalence of metabolic diseases also has increased with greater dietary fat consumption (Hooper *et al.* 2001). The quantity and quality of the fat in the diet has changed in the National Diet Surveys over the last three decades in the UK. However, there are large variations in the amount of total fat intake across countries in different parts of the world (FAO/WHO, 2003). The assessment of fat intake and subsequent CHD mortality in a cohort of randomly selected adults aged 40-75 years old for 16 years since1984-1985 up to 2000-2001 in the UK showed there is a positive and significant relationship between fat intake and mortality from CHD while taking account of other CHD related risk factors (Boniface and Tefft 2002), however the cross-sectional Scottish Heart Health Study (Bolton-Smith *et al.* 1992) did not find any association in males or females.

There is strong evidence for the link between fat intake and risk of chronic diseases such as CVD in experimental animals. Studies in animal models of atherosclerosis in hamsters or transgenic models of atherosclerosis in mice, (Mangiapane *et al.* 1999) indicated the contribution of a high saturated fat diet to CVD disorders. Atherogenic processes were also seen in healthy human subjects after a high saturated fat diet (Hu *et al.* 2001) and large scale epidemiologic studies confirm these associations (Hooper *et al.* 2001). Reduction in total fat intake and especially intake of saturated fatty acids is an important aspect of dietary guidelines, particularly in Western countries. The recommended intake for dietary fat in the UK is 33% of total energy (including alcohol) and 35% of food energy (excluding alcohol) (Table 1.5). The proportion of saturated fat should be around one third of energy from fat (i.e. about 10% of total dietary energy). Mono- and poly- unsaturated fatty acids should provide on average about 12% and 6% of dietary energy, respectively. The proportion of saturated fatty acids should not exceed 10% of total energy (Department of Health, 1991). Storage lipid plants (e.g. vegetable oils) and marine animals (e.g. fish) are good dietary sources of fat.

Figure 1.13 Geometric configuration of unsaturated fatty acids.

Vegetables, cereal products and fat spreads are main sources of n-3 and n-6 fatty acids in the diet of British adults (Gregory *et al.* 1990). The other important issue is the geometric nature of the consumed unsaturated fatty acids (Constant, 2004).

Figure 1.13 shows the geometric structure of two different conformations of unsaturated fatty acids present in human metabolism. The production of the trans form of unsaturated fatty acids (mono, di and poly unsaturated fatty acids) by thermo reactive processes results in a higher melting point compared with the isomeric compound of the same fatty acid in cis configuration (van Greevenbroek *et al.*1998; Wang *et al.* 2003). This will affect membrane fluidity and response to hormones.

Several large-scale studies report trans fatty acids as a significant risk factor for CHD (Lichtenstein *et al.* 2003), in contrast, some smaller scale studies did not reveal any association with insulin resistance (Lovejoy *et al.* 2002).

1.6 Effects of high carbohydrate (HC) diets

As a high carbohydrate diet is recommended for the whole population, it is important to consider the scientific basis on which these recommendations were made and whether the recommendation is still relevant.

The interaction between dietary carbohydrate and lipid metabolism in health and disease has been a major research interest since Ruderman *et al.* (1971) reported an increase in plasma TAG levels on a high carbohydrate diet. Disturbances in carbohydrate metabolism characterised by high glucose concentrations and other features of insulin resistance are more likely to be accompanied by dyslipidemia (Abbasi *et al.* 2000; Parks and Hellerstein, 2000; Bessesen, 2001, Isomaa *et al.* 2001; Hellerstein, 2002; Thomas and Wolever, 2003). In 80% of patients with type 2

diabetes, metabolic syndrome (i.e. obesity, dyslipidemia, hypertension, and microalbuminuria) is observed (Bos et al, 2003). The presence of insulin resistance and metabolic syndrome in type 2 diabetic patients results in hypertriglyceridemia and low HDL-cholesterol concentrations and high levels of small dense LDL particles and consequently a higher risk of atherosclerosis (Goldberg, 2000).

The health effects of increasing carbohydrate intake through decreasing dietary fat have been investigated through various epidemiological and intervention studies. Although there is no universally accepted definition for HC and low carbohydrate (LC) diet according to either percentage of energy intake derived from carbohydrate or absolute amount of carbohydrate in the diet, most studies have defined HC diet as a diet with 55% or more energy from different types of carbohydrate (Jeppesen *et al.* 1997). Some aspects, such as the difference between high or low carbohydrate diets in isoenergetic conditions as well as different level of energy intake, amount of dietary carbohydrate (gradient effect), various chemical forms of carbohydrates (simple or complex), rate of carbohydrate digestibility, quality of carbohydrate (glycaemic index of food), duration of study (short- and long- term), in animal models and human, in healthy and patients subjects (particularly type 2 diabetic patients), interaction by genetic and lifestyle factors (e.g. physical activity level) have been of concern. Although some studies revealed variability in the response to HC diet, health benefits such as lower risk of CHD and type 2 diabetes, weight loss, reduction in total and LDL-cholesterol concentrations have been reported.

1.6.1 Beneficial effects of HC diets

The LF-HC diets might play a role in weight loss associated with improvements in glucose metabolism and insulin sensitivity. Diets higher in unrefined carbohydrates and dietary fibre have been shown to slow down glycaemic and insulinaemic responses compared with refined carbohydrate diets. For example, increased whole-grain intake was associated with decreased risk of CHD after adjustment for age and smoking (Liu *et al.* 1999). Therefore, such diets may protect from the development of type 2 diabetes and related health outcomes (Hu *et al.* 2001b). Dietary patterns including higher intakes of fruits, vegetables, legumes, fish, poultry, and whole grains, a so-called 'prudent' dietary pattern, has been shown to be associated with a lower risk for CHD (RR=0.76, 95%CI: 0.60-0.98) compared with the 'Western dietary pattern' characterised by higher intakes of red and processed meats, sweets and desserts, french fries, and refined grains which significantly increased the risk of CHD (Fung *et al.* 2001). The protective effects against CHD have been shown by increasing consumption of fruits and vegetables, particularly green leafy vegetables and vitamin C-rich types (RR=0.80, 95% CI: 0.69 to 0.93; Joshipura *et al.* 2001).

The HC-LF diet including at least five portions of fruit and vegetables per day is supposed to reduce risk of CHD (Ullmann *et al.* 1991; Rimm *et al.* 1996; Dreon *et al.* 1999; Liu *et al.* 1999; Joshipura *et al.* 2001; Cernea *et al.* 2003). Reductions in body weight, total cholesterol, LDL and HDL cholesterol concentrations were shown following the American Heart Association Step 1 diet, as a HC-LF and low cholesterol, after 10 weeks among overweight and obese women (Bunyard *et al.* 2002). However, a two-week isoenergetic HC and HF intervention diets (i.e. 75% and 10% *vs* 30% and 55% energy from carbohydrate and fat, respectively) failed to show any significant difference in plasma total cholesterol concentration

(Mittendorfer and Sidossis, 2001). Variability in response to HC diet among healthy people could be a major factor (Ruderman *et al.* 1971; Mancini *et al.* 1973; Parks *et al.* 1999; Parks and Hellerstein, 2000). In addition, different types of dietary carbohydrates have different glucose and insulin responses (Wolever *et al.* 1988; Riccardi *et al.* 2003), and consequently, may result in different metabolic effects (Laville, 2004).

1.6.2 Adverse effects of HC diets

Reduction in fat intake (at 33%) and its replacement by carbohydrate in which energy intake is maintained at the same level is recommended in the UK. However, this recommendation does not seem to reduce the risk of CHD. Despite the potential beneficial effects of a HC diet, there is evidence indicating that HC diet leads to increases in plasma TAG and a decrease in HDL cholesterol concentrations (Chen *et al.* 1993; Jeppesen *et al.* 1997; Koutsari *et al.* 2000). Hypertriglyceridemia as a predictor of CHD risk has been reported through intense studies after following not only a HF diet but also HC diet (Kasim-Karakas *et al.* 1997; Austin, 1999; Parks *et al.* 1999; Hudgins *et al.* 2000; Kasim-Karakas *et al.* 2000; Parks and Hellerstein 2000; Parks, 2001; Hellerstein, 2002). The increase in TAG levels, however, has been associated with a reduction in LDL particle size (Kasim-Karakas *et al.* 1997).

Short-term intervention studies have similarly reported hypertriglyceridemia and reduction in HDL-cholesterol levels. The effect of two experimental isoenergetic diets: (1) HC diet (60% of energy from carbohydrate and 25% from fat) and (2) HF diet (40% and 45% energy from carbohydrate and fat, respectively) for three weeks, showed higher fasting TAG and VLDL cholesterol concentrations and lower HDL

cholesterol level after the HC diet compared with the HF diet (Kasim-Karakas *et al.* 1997).

In order to maintain glucose homeostasis, an increase in carbohydrate intake imposes a demand for insulin secretion. An increase in TAG and a decrease in HDL-cholesterol concentrations have been reported following diets high in either carbohydrate or polyunsaturated fat (Brunner *et al.* 2001). As HC diet is one of the reasons for hypertriglyceridemia, there has been an interest to find the best type of carbohydrate to avoid disturbances in glycemia. Results of various studies have shown that, high amylopectin starch (Kabir *et al.* 1998) and wheat starch (Lever-Metzger *et al.* 1996) induce hyperglycemia, hyperinsulinemia and hypertriglyceridemia as well as increasing adiposity in normal and diabetic rats.

The major metabolic pathway for supply of energy reserves and cellular structure is fatty acid synthesis, which is regulated by diet (e.g. HC and low-fat (LF) diets) and hormonal controls (e.g. insulin, glucagon and thyroid hormones) (Hillgartner et al, 1995). For instance, three week consumption of a high GI diet may lead to adverse effects on lipogenic enzymes (i.e. fatty acid synthase and LPL) to increase plasma lipids and fat accumulation while low GI diets (low in amylopectin and high in amylose) inhibits long-term fat accumulation by inhibition of these lipogenic enzymes in normal and diabetic rats (Kabir *et al.* 1998).

De novo lipogenesis has been proposed as one of the probable mechanisms to explain dyslipidemia following a HC diet. *De novo* lipogenesis is defined as the process by which carbon units from carbohydrates are used for fat synthesis (Parks,

2001). Fatty acids synthesised from dietary triglycerides, sugars from consumed carbohydrates, adipose tissue or remnant TAG following the action of LPL on chylomicrons are the sources of VLDL-TAG synthesis in the liver (Figure 1.14).

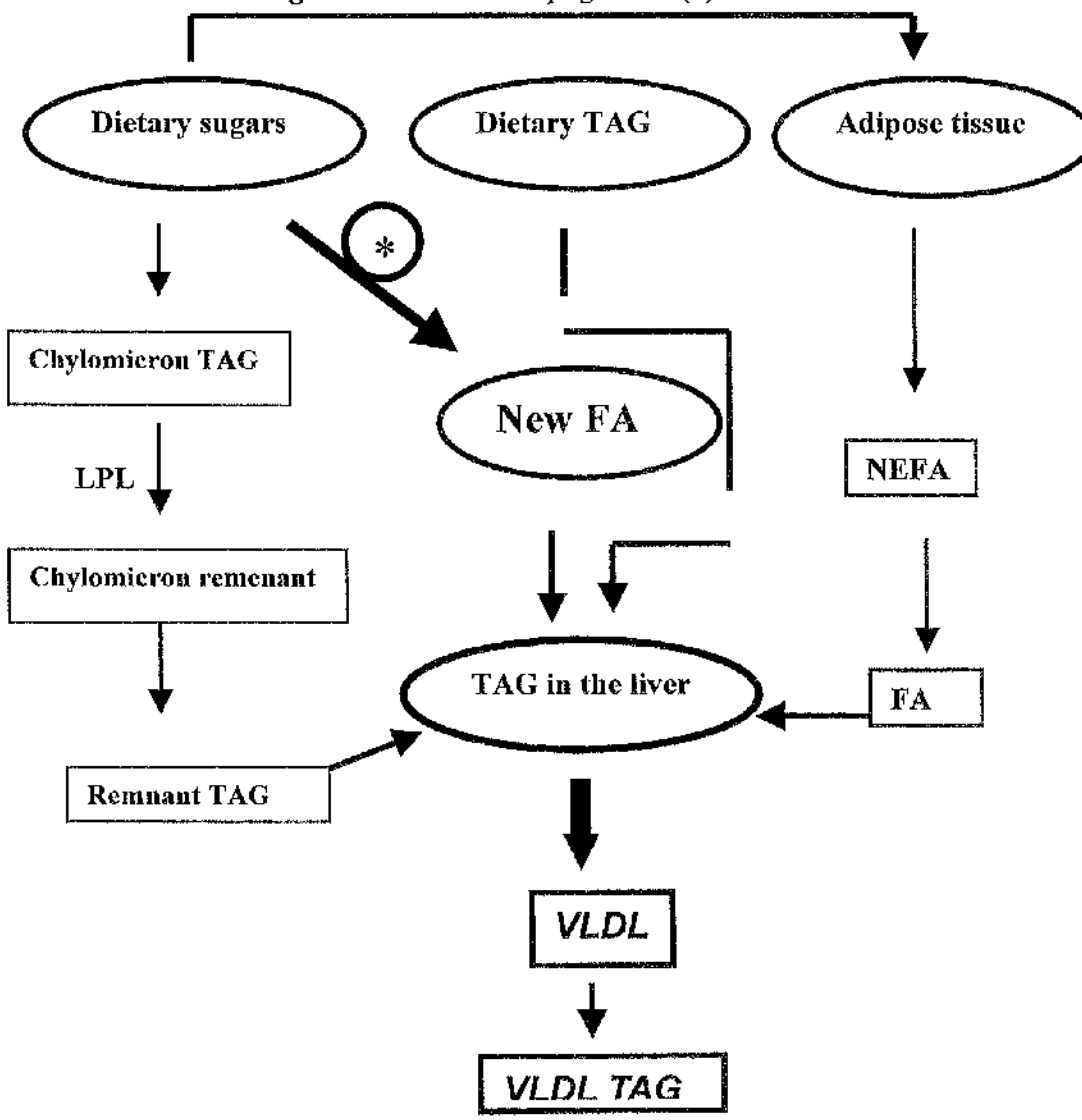
Hudgins *et al.* (2000) showed that diets high in simple sugars were significantly associated with higher *de novo* lipogenesis in healthy subjects. This condition may stimulate VLDL-TAG synthesis but little is known about stimulation of fatty acid synthesis in humans. However, results of the *in vivo* measurements of fatty acid synthesis have revealed that despite high body carbohydrate stores and stimulation of *de novo* lipogenesis by HC diet in humans, fatty acid synthesis through this process is quantitatively minor (Hellerstein *et al.* 1996). One of the possible hypotheses is that high monosaccharide diets increase blood glucose levels and are more likely increase fatty acid synthesis from glucose by providing more carbon units to the liver. Therefore, elevated levels of glucose and insulin may stimulate *de novo* lipogenesis. Parks (2002) failed to find any positive association between these parameters among subjects followed HC diets but there was a positive relationship between insulin concentration and *de novo* lipogenesis in subjects on HF diet (Figure 1.15). Non-digestible oligosaccharides (e.g. oligofructose) may directly influence increased lipogenesis. Oligofructose is fermented in the colon and provides short-chain fatty acids (SCFA). It has been suggested that TAG and cholesterol synthesis are affected by the proportion of propionic to acetic acid. In one study, supplementation with 15g/day oligofructose in healthy subjects reduced fasting TAG level by 21% (Parks, 2002).

Proposed factors influencing the control of liver TAG secretion are:

- the form of carbohydrate (solid or liquid),
- the level of carbohydrate processing in food,
- the physical state of the carbohydrate in the diet

(Parks & Hellerstein 2000; Parks 2002).

Figure 1.14 *De novo* lipogenesis (*)



In general, these studies are mostly very short term and the effects may not be sustained over a longer time. There are arguments for appropriate amounts of

carbohydrate and fat in daily energy intake (Hung *et al.* 2003). The controversy for dietary fat and carbohydrate for the management of diabetes has produced three main guidelines for the management of diabetes since 2000.

In the first approach, saturated fatty acids (SF) were avoided and it was emphasised to provide energy from carbohydrate and monounsaturated fatty acids (MUFA), however there was no regard to carbohydrate quality in the recommendations of the American Diabetes Association (ADA). The most recent ADA advice is for for diabetics to reduce body weight and increase physical activity. with 60-70% of energy from MUFA and complex carbohydrate with no attention to the quality of carbohydrate (Parks, 2002).

Nevertheless, there is a special interest in the quality of carbohydrate (e.g. glycaemic index). Low glycaemic index (low GI) and high fibre carbohydrates are recommended and this type of dietary carbohydrate is considered as an important parameter for diabetes management. The Canadian Diabetes Association (CDA) and the Diabetes Australia (DA) have also recommended low fat, low saturated fat and high carbohydrate diet (FAO/WHO, 1997).

The European Association for the Study of Diabetes (EASD) has combined the two approaches (The Diabetes and Nutrition Study Group (DNSG) of European Association for the study of Diabetes, 2000). The American Heart Association (AHA) in 2000 and National Cholesterol Education Programme (NCEP) in the third Adult Treatment Panel (ATP-III) in 2001 (LaRosa and Gotto, 2004) (Isomaa *et al.* 2001; Isomaa, 2003) have addressed nutritional management of diabetes and insulin

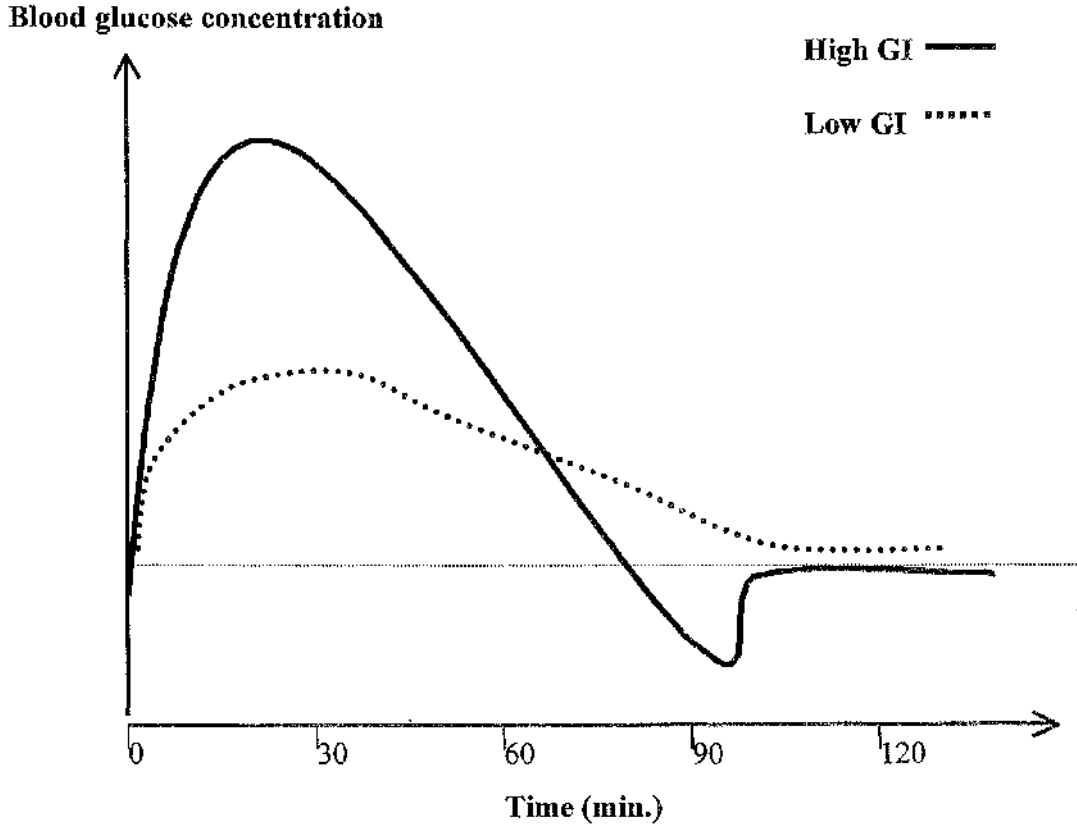
resistance and they recommend the modification of carbohydrate quality by an increase in dietary fibre and MUFA up to 20% of daily energy intake.

1.7 Glycaemic index (GI)

The concept of Glycaemic Index is an extension of the dietary fibre theory that was introduced by Burkitt and Trowell in 1977. Consumption of fibre reduces the rate of nutrients influx from intestine (Jenkins *et al.* 2002). The rate of digestion contributes to the postprandial blood glucose and secreted insulin in response to the dietary intake of carbohydrate containing foods (Wolever and Bolognesi 1996).

In order to guide food choices in 1997, a committee of experts commissioned by the FAO and the WHO reviewed the research evidence regarding the importance of carbohydrates in human nutrition and health. They accepted the use of glycaemic index (GI) method to classify foods rich in carbohydrate and that to consume from carbohydrates with low GI is good for health. A low GI diet can reduce postprandial glucose levels and keep blood glucose levels within a normal range (Brynes *et al.* 2005) which may increase insulin sensitivity.

Figure 1.15 Blood glucose response to low and high GI foods up to two



Jenkins (1981) proposed that the accepted categories in the classification of dietary carbohydrate and the division of simple and complex carbohydrates could not explain all the characteristics and observations of the physiological effects of different carbohydrate consumption especially in diabetics (Opperman *et al.* 2004). Jenkins introduced the GI theory in 1981 and since then has carried out much research into this concept and th application of this new classification in understanding of carbohydrates in the diet of diabetic patients and especially in the postprandial condition.

1.7.1 Definition of GI

The GI of a food can provide useful information on the likely effect of a carbohydrate-containing food on postprandial blood glucose. It is based on the area under the postprandial blood glucose curve after enough of the food to contain 50g of carbohydrate compared with 50g of readily digestible carbohydrate. A syrup of glucose or white bread is used as the standard digestible carbohydrate. The GI is expressed as a percentage of the glycaemic response to the reference food. Figure 1.15 shows the difference in glycaemic response between foods with high and low GI. The GI of a food does not reflect the quantity of carbohydrate in the food. If a food contains very little carbohydrate you need to eat a large volume to carry out the measurement, which may affect the result.

1.7.2 Definition of glycaemic load (GL)

The term glycaemic load (GL) quantifies the average glycaemic effect of a portion of food and was introduced in 1997 (Frost *et al.* 1999; Liu *et al.* 2000; Jenkins *et al.* 2000b; Laville, 2004). The GI of a carbohydrate containing food provides information on the nature of food while the GL reflects the resultant blood glucose and insulin level (Nantel, 2003). The GL is the product of the amount of available carbohydrate and the GI of the food and is calculated by multiplying GI of the food by carbohydrate content of the food divided by 100 (Foster-Powell *et al.* 2002).

1.7.3 Calculation of average GI and GL

Average GI and GL are calculated by either direct assessment of GI for foods or use of previously determined and published values for each food. For determination of average GI and GL values, dietary data should be analysed. To obtain carbohydrate

content for each food, a list of the consumed food items is provided and then carbohydrate content is estimated. The GI for each food is selected appropriately from the International Table of Glycemic Index and Glycemic Load Values (Foster-Powell *et al.* 2002). The average GI in the table is based on studies in either healthy or diabetic subjects (type 1 or type 2 diabetes) and foods have been sorted descending according to their carbohydrate content. It has been shown that GI values determined in normal subjects correlate well with those found in diabetic subjects (Wolever *et al.* 1987). Even though people with diabetes have higher blood glucose values, they are members of the same human species and they show the same differences in rates of digestion and absorption of carbohydrate (Brand-Miller and Mendosa, personal communication on 17, August 2003). For food items with high carbohydrate content and without GI, it is recommended to consider the GI from similar or the closest food item by type and amount of carbohydrate. It is also suggested that if 80% of the consumed food items with GI value from the top of the list are prepared, it could be enough for calculation of GL and average GI (Jenkins 2003, the 9th European Congress on Nutrition, Rome).

1.7 4 Advantages and disadvantages of GI and GL

There are several advantages and disadvantages to the use of GI and GL. A physiological classification of dietary carbohydrates in common foods has an important advantage over the traditional classification using molecule size. Classification of a food based on its glycaemic response provides an indication of the carbohydrate quality (Schenk *et al.* 2003) and also a reasonable insight into the link between foods and health outcomes. GI has been shown to be a strong predictor of HDL-C and C-reactive protein levels in healthy and patients with chronic disease

such as metabolic syndrome, CHD, obesity and some kinds of cancer. (Brand-Miller *et al.* 2003; Frost et al 1999; Liu et al 2000). However, many factors such as food form, cooking method, processing and structure of starch have a strong influence on the GI of foods. There can be a great variability of the GI of foods depending on the botanic origins of the food, the time and temperature during cooking, and the fat and water content (Brand-Miller *et al.* 2003; Laville 2004). Moreover the GI of a food does not reflect the real postprandial glycaemia of the normal portion size of the food in conjunction with other meal ingredients. The GL improves on this but still does not necessarily reflect the true postprandial consequences of the diet.

The determination of the postprandial response for each food, which is required by both GI and GL, is expensive and in general people use the published international table of GI and GL values instead of measuring their own foods (Sydney University-Glycaemic Index Research Service 2003). This means that many studies may under or overestimate the response to the foods in their country's diet. Table 1.9 and 1.10 summarise advantages and disadvantages of application of GI and GL. Table 1.11 presents some examples of food with different GI and GL.

Table 1.9 Advantages and disadvantages of Glycaemic index (GI)

Advantage	Reference	Disadvantage	Reference
Physiologically classifying carbohydrate-containing foods, to assess glycaemic response compared with the reference food (glucose or white bread).	*Schenk, 2003*	Many factors such as food form, cooking method, processing, starch structure and particle size affect the GI. There is a great variability in the GI depending on the origin of food and the way of cooking (time, temperature, water content and etc...).	*Wolever, 2003* *Laville 2004*
An indicator of carbohydrate quality	*Foster-Powell (2002)*	Expensive to determine GI value for a given food using a valid methods Not an indicator of carbohydrate quantity	*Sydney University Glycemic Index Research Service (2003)*
Better insight in link between food and health outcomes (e.g. predictor of HDL-C and CRP levels) in healthy population and people with chronic diseases (eg type 2 diabetes mellitus, metabolic syndrome, CHD, obesity, colon, ovary and breast cancer).	*Ridker (2002)* *Brand-Miller (2003)*		
International table of glycaemic index and glycaemic load values, a reliable source to evaluate GI and GL values	*Foster-Powell (2002)*	Lack of GI values for a number of carbohydrate containing food items	*Jenkins (2002c)*
Independent predictor of risk of type 2 diabetes, CHD and high level of CRP	*Brand-Miller (2003)* *Ridker (2002)*		

Table 1.10 Advantages and disadvantages of Glycaemic load (GL)

Advantage	Reference	Disadvantage
GL value, the product of the GI of specific foods and their carbohydrate content, takes the amount of carbohydrate intake into account and gives fuller picture of carbohydrate type and content than does GI. Therefore, it reflects actual carbohydrate burden.	*Brand-Miller (2003)* *Foster-Powell (2002)*	Accurate weighed-food record is required.
Surrogate measure of the ability of a meal to induce hyperglycemia, hyperinsulinemia, hypertriglyceridemi, lower HDL-C concentrations and higher level of CRP concentrations	*Ridker (2002)* *Brand-Miller (2003)*	
It could be calculated by a validated semi- quantitative food frequency questionnaire.	*Liu (2001)*	
	Opperman (2004)	The only published meta-analysis of GI and GL is on diabetic patients not healthy subjects.

Table 1.11 Glycaemic Index by Glycaemic Load (Mendosa 2004)

	LOW GI	MED GI	HI GI
LOW GL	All-bran cereal (8*,42**)	Beets (5,64)	Popcorn (8,72)
	Apples (6,38)	Cantaloupe (4,65)	Watermelon (4,72)
	Carrots (3,47)	Pineapple (7,59)	Whole wheat flour bread (9,71)
	Chana dal (3,8)	Sucrose (table sugar) (7,68)	
	Chick peas (8,28)		
	Grapes (8,46)		
	Kidney beans (7,28)		
	Nopal (0,7)		
	Oranges (5,42)		
	Peaches (5,42)		
	Peanuts (1,14)		
	Pears (4,38)		
	Pinto beans (0,39)		
	Red lentils (5,26)		
	Strawberries (1,40)		
	Sweet corn (9,54)		
MED GL	Apple juice (11,40)	Life cereal (16,66)	Cheerios (15,74)
	Bananas (12,52)	New potatoes (12,57)	Shredded wheat (15,75)
	Buckwheat (16,54)	Wild rice (18,57)	White wheat flour bread (11,70)
	Fettucine (18,40)		
	Navy beans (12,38)		
	Orange juice (12,50)		
	Parboiled rice (17,47)		
	Pearled barley (11,25)		
	Sourdough wheat bread (15,54)		
III GL	Linguine (23,52)	Couscous (23,65)	Baked Russet potatoes (26,85)
	Macaroni (23,47)	Sweet potatoes (27,61)	Cornflakes (21,81)
	Spaghetti (20,42)	White rice (23,64)	

Adapted from Foster Powell *et al.*, 2002; Mendosa, 2004

GI: Low=1-55 Med=56-69 High=70-100

GL: Low=1-10 Med=11-19 High=20 or more

*** Food GL**

**** Food GI**

GL (g) =((GI (%) / 100)) * Carbohydrate (g))

There is not a general agreement about the importance of GI on human health, nutrition and disease prevention (Ludwig and Eckel, 2002; Kelly et al 2004). Table 1.12 summarises the name and references of organizations that did accept or did not encourage application of GI parameter as a base for dietary managment. It is therefore important to consider the epidemiological evidence for the importance of GI and GL in the incidence of chronic disease.

Table 1.12 **Approval and disapproval of GI by different organizations in the world (Opperman et al. 2004).**

Organization approved use of GI	**References**	**Organization disapproved use of GI**	**References**
Joint FAO/WHO Expert Consultation on Carbohydrates	1997	American Diabetes Association	2001
European Association for the study of Diabetes (EASD)	2000	American Heart Association	Krauss *et al.* 2000
Canadian Diabetes Association	2000	American Dietetic Association	1999
Diabetes UK	2003		
Dietitians Association of Australia	1997		

1.8 Association between dietary GI and GL and chronic disease

There are several epidemiological studies which have investigated the association between GI, GL and chronic diseases including cardiovascular diseases, cancer and type 2 diabetes (Ludwig, 2003c; Tables 1.13, 1.14) GL has been suggested as an

independent risk factor for myocardial infarction (Ford and Liu, 2001; Liu *et al.* 2001), cancer (Augustin *et al.* 2001; Augustin *et al.* 2003) and type 2 diabetes (Salmeron *et al.* 1997a; Salmeron *et al.* 1997b).

Liu *et al.* examined the association between dietary GL and risk of coronary heart disease (CHD) in 75,000 women aged 38-63 years in 1984 and after 10 years follow-up using a validated food frequency questionnaire. Over 10 years follow-up, 208 fatal and 553 non-fatal myocardial infarction occurred. After adjustment for other CHD risk factors (e.g. age, smoking, total energy intake), a high dietary GL was associated with a higher risk of CHD (Liu *et al.* 2001).

The first cross-sectional study in UK investigated the relationship between dietary GI and cardiac risk factor such as HDL cholesterol, was carried out by Frost *et al.* in 1999. The retrospective study was undertaken on 7-day weighed records of 1,420 British adults. Results showed that there was a negative correlation between dietary GI and serum HDL cholesterol concentrations (Frost *et al.* 1999). Ford and Liu (Ford and Liu, 2001) in the US population also confirmed this finding. They reported that plasma HDL level was inversely associated with dietary GI after adjustment for risk factors for CHD, that is the HDL level for the lowest GI quintile was 1.36 $mmol.L^{-1}$ while its concentration was 1.26 $mmol.L^{-1}$ in the highest quintile of GI.

1.8.1 GI and GL and Type 2 diabetes mellitus

Diet is one of the pivotal factors which influences the development of type 2 diabetes (Hu *et al.* 2001a; van Dam *et al.* 2002b). HC-LF diets might aid in weight loss associated with improvements in glucose metabolism and insulin sensitivity.

Complex and unrefined carbohydrates cause slower glycaemic and insulinaemic responses compared with highly processed refined grains and in this way play a protective effect on the development of type 2 diabetes (Meyer *et al.* 2000; Hu *et al.* 2001b).

Dietary GI has been used as a carbohydrate quality indicator for studies investigating the link between dietary carbohydrate and the risk of type 2 diabetes. The link between dietary GI and GL in relation to lipid profile has mostly been studied with diabetic patients. Dietary carbohydrates may influence the risk of developing type 2 diabetes. For example, there is strong evidence indicating the association between lower dietary fibre intake and an increased risk of developing type 2 diabetes (Salmeron *et al.* 1997a; Salmeron *et al.* 1997b; Meyer *et al.* 2000) whereas a few studies have reported no association between total carbohydrate intake and diabetes risk (Salmeron *et al.* 1997a; Salmeron *et al.* 1997b; Meyer *et al.* 2000).

A number of epidemiological studies have investigated the association between the GI or GL of diet and diabetes risk using multivariate adjusted relative risk (RR). For example, the Nurses' Health Study has revealed that increase in GI of diet was positively associated with risk of type 2 diabetes after 6 years follow-up. The adjusted RR for women was 1.37 (95% CI: 1.09, 1.71) for an increase in GI of 15 units and 1.47 (95% CI: 1.16, 1.86) for the highest quintile of dietary GL. Women with both a high GL and a low cereal fibre intake were at an even higher risk of type 2 diabetes (2.43; 95% CI: 1.12, 5.27) (Salmeron *et al.* 1997a). Among men, the RR was 1.37 (95% CI; 1.02, 1.83) for extreme quintiles of dietary GL and 2.17 (95% CI: 1.04, 4.54) for the combination of a high GL and a low intake of cereal fibre

(Salmeron *et al.* 1997b). However, the Iowa Women's Health Study cohort failed to find any associations between GI and GL and diabetes risk in the six-year follow-up (Meyer *et al.* 2000).

1.8.2 GI and GL and Coronary heart disease

Dietary GI and GL are suggested as important dietary indicators in relation to lipid metabolism and insulin-related disturbances and consequently CHD risk (Wolever *et al.* 1991; Frost *et al.* 1999; Liu *et al.* 2000; Jenkins *et al.* 2000a; Laville, 2004). These indicators have mainly been studied by metabolic risk factors for CHD rather than assessing the direct effect on incidence of CHD. The most frequently studied risk factors of CHD are lipid profile, (e.g. TAG, total, HDL and LDL cholesterol), insulin-related parameters (e.g. glucose, insulin, and insulin sensitivity index) and inflammatory markers (e.g. C-reactive protein).

1.8.2.1 Lipid risk factors

These associations have been examined through epidemiological and intervention studies. High-GI diet has been shown to be associated more with higher CHD risk (Frost *et al.* 1999; Jenkins *et al.* 2000a). Dietary GI is associated positively with TAG concentrations, negatively with HDL levels (Liu *et al.* 2000), inversely associated with satiety through short-term feeding and with weight loss (Leonetti *et al.* 2002; Pawlack *et al.* 2004). Food form, particle size, food processing and cooking and structure of carbohydrate affect the GI. In addition, glucose and insulin responses vary by amount and type of carbohydrate (Wolever *et al.* 1988; Brand-Miller *et al.* 2003) and various metabolic effects could be expected (Laville, 2004). Slow release of insulin as a result of slowly absorbable carbohydrate seems to

modulate lipid concentrations (Riccardi *et al.* 2003). Increase in carbohydrate intake and particularly higher intake of simple sugars than starches can induce hypertriglyceridemia. Plasma TAG levels increase in a dose-dependent manner (Leeds, 2002; Ludwig, 2002; Brand-Miller *et al.* 2003; Fried and Rao, 2003). Low GI carbohydrates is supposed to improve lipid profile in hyperlipidemic patients by increasing HDL cholesterol concentrations because they are slowly absorbed and form lente or sustained release carbohydrates by providing a substrate for colonic fermentation (Jenkins *et al.* 2002).

However, not only no protective effect of dietary GI has been shown on lipid profile and risk of acute myocardial infarction (Tavani *et al.* 2003; Frost *et al.* 2004) or even increased risk of CHD (Brynes *et al.* 2003). The most important effects of GI on lipid profile are low plasma HDL concentrations and elevated fasting TAG concentrations (Liu *et al.* 2000; Liu *et al.* 2001). Among 280 postmenopausal women in the Nurses' Health Study, dietary GL was associated with low plasma HDL cholesterol and elevated fasting TAG concentrations (Liu *et al.* 2001). A similar effect on HDL cholesterol was found by Frost *et al.* (Frost *et al.* 1999) on British healthy adults.

Results from a meta-analysis of 17 population-based studies showed a 76% increase in CVD risk in women and a 31% increase in men associated with a 1 mmol/L increase in TAG levels (Opperman *et al.* 2004). It could be partially explained by hormonal difference between women and men as menstrual cycle has an important influence on TAG levels but not on cholesterol concentrations. The risk of CHD increases after menopause (Rich-Edwards *et al.* 1995; Tremollieres *et al.* 1996; Jeppesen *et al.* 1997; Woods *et al.* 1998).

Insulin seems to be a good predictor for HDL cholesterol concentrations in healthy populations and diabetic patients whereas type and amount of fat failed to show it (Ford *et al.*1999). Insulin is at the centre of metabolism of ingested nutrients, in particular dietary carbohydrate. Insulin by reducing gluconeogenesis, glycogenolysis and suppressing lipolysis and the release of NEFA, stimulates the disposal of ingested glucose into muscle and adipose tissue (Frayn *et al.* 1997; Kraegen *et al.* 2001).

Low GI diet has shown to improve insulin sensitivity while high GI diet is supposed to be associated with insulin resistance (Salmeron *et al.* 1997a; Salmeron *et al.* 1997b; Ludwig, 2002). In addition, LPL is activated by insulin in adipose tissue. Thus, in postprandial condition, because of an increase in TAG level, the clearance of TAG-containing lipoprotein increases. The mechanisms for hypertriglyceridemia following consumption of high GI diet are what were described earlier in the effect of HC diet. Because there is a strong interrelationship between TAG and HDL cholesterol concentrations (Figure 1.4 and 1.5), similar mechanisms are supposed for HDL cholesterol as well.

1.8.2.2 Insulin-related parameters

Insulin resistance is a metabolic disorder and associated with obesity, hypertension, diabetes and CHD. Alteration in diet is supposed to influence insulin sensitivity however, other factors may contribute to its progression. Associations between insulin resistance and disease such as CHD have observed in some but not all populations (Bessesen, 2001). Plasma glucose, insulin, and insulin sensitivity indicators (such as homostatic model assessment (HOMA) or euglycemic

hyperinsulinemic clamp technique) are a number of insulin-related factors. Although large-scale epidemiological studies such as the Health Professional Follow-up Study, the Iowa Women's Health study and the Nurses Health Study failed to establish the association between diet composition and diabetes (Salmeron *et al.* 1997a; Salmeron *et al.* 1997b; Meyer *et al.* 2000); results of studies have shown that HC diet does not adversely influence insulin sensitivity compared with HF diet (Jeppesen *et al.* 1997; Bessesen, 2001).

Low GI diet has shown to improve insulin sensitivity (Ludwig *et al.* 1999) and reduce the risk of type 2 diabetes (Salmeron *et al.* 1997a; Salmeron *et al.* 1997b). Nevertheless, using the euglycemic hyperinsulinemic clamp as a gold standard technique for measuring insulin sensitivity failed to show the association with dietary GI in healthy and highly active young men (Wolever, 2000). Low GI starches seem to improve insulin sensitivity but variations in the type, amount and rate of absorption of dietary carbohydrate are other determinants of glucose and insulin responses (Lineback and Miller Jones, 2003). Four-week consumption of low GI diet resulted in reduction in area under the glycaemic response curve (AUC) in response to oral glucose and improved insulin sensitivity among patients with CHD (Frost *et al.* 1996). In another study of Frost *et al.*, they found that low GI diet improved in vitro insulin sensitivity measured by reduction in glucose after insulin injection (Frost *et al.* 1998) and also decreased HDL cholesterol concentration following low GI diet was due to the improving effect of low GI diet in insulin sensitivity (Frost *et al.* 1999). Similar results in HDL level was also found by one month following HC-high GI diet in type 2 diabetic patients (Luscombe *et al.* 1999).

Table 1.13 Epidemiologic studies that assessed the contribution of GI and GL to the metabolic risk factors for CHD

Study	Subjects	Observations	Effects	Comments	References
Prospective evaluation of contribution of GL & dietary fibre to risk of NIDDM in Nurses' Health Study	65,173 US female nurses (30-55 years old) free of disease, monitored 6 y by FFQ during follow-up, controlling for major risk factors for NIDDM.	Positive association of GI with risk for NIDDM after adjustment for age, BMI, smoking, physical activity, genetic background, cereal fibre & total energy, RR of NIDDM was 1.37 (CI 1.09-1.71 P=0.005).	RR for cereal fibre 0.72 (%95 CI 0.58-0.90 P=0.001) NIDDM risk Combination of high GL & low cereal fibre intake increased risk for NIDDM (RR=2.50 95% CI, 1.14-5.51) compared with low GL & cereal fibre intake.	Large sample size, only women. Evaluation of the consumed GI & consequently the calculated GL limits the validity of result. Subjects all nurses and not representative of US society.	Salmeron *et al.* 1997a
Prospectively evaluated contribution of GL to risk of NIDDM among healthy men in US Health Professionals Follow-up Study	42 759 men without NIDDM or CHD (40-75 years old) studied by FFQ for 6 years, controlling for major common and known risk factors.	Positive association between risk of NIDDM after adjustment for age, BMI, smoking, physical activity, genetic background, cereal fibre & total energy, RR of NIDDM was 1.37 (CI= 1.02-1.83 P=0.03).	RR for cereal fibre 0.70 (% 95CI, 0.51-0.96 P=0.007 for NIDDM risk. Combination of high GL & low cereal fibre intake increased risk for NIDDM (RR=2.17 95% CI , 1.04-4.54) compared with low GL & cereal fibre intake.	Large sample size, only men. Evaluation of the consumed GI & consequently the calculated GL. makes results questionable. Subjects do not represent US society.	Salmeron *et al.* 1997b
Cross-Sectional study of British Adults (16-64 years) assessed individuals at increased risk of CHD	1420 participants (male & female) with dietary & biochemical data.	Reverse & significant association between dietary GI and HDL-C level.	Dietary GI predicted circulating HDL level & was stronger predictor than dietary fat intake of HDL-C.	GI of the diet does not show the carbohydrate load of the consumed diet.	Frost *et al.* 1999
Cross-Sectional study among US adults	GI & GL were calculated by FFQ in 13 907 participants aged 20 years and older in the Third National Health and Nutrition Examination Survey (1988-1994) in the US	Reverse association between HDL level & GI or GL values	Predictive value of GI or GL to assess the risk of CHD	Cross-sectional methodology	Ford and Liu, 2001
Women's Health Study (WHS), double-blind, placebo-controled trial, assessesed low dose Asprin & vitamin E in prevention of CVD & cancer	244 apparently middle-aged healthy women in the US by SFFQ. Average GL assessed & plasma hs-CRP determined.	Positive & strong association between GL & hs-CRP		Cross-sectional study	Liu *et al.* 2002
Prospective assessment of the association between GI & GL & dietary fibre with risk of type 2 diabetes in women (The Nurses' Health Study II) in the US	91 249 women using semi quantitative FFQ to assess dietary intake for 8 years followed for development of type 2 diabetes.	Significant association of GI with an increased risk of diabetes, but GL does not. Cereal fibre intake was associated with a decreased risk of diabetes	Positive association between GI and type 2 diabetes & protective association with cereal intake.	Dietary GL is the real indicator for quality of diet because type of carbohydrate & amount of the consumed carbohydrate would be taken in account.	Schulze *et al.* 2004

Table 1.14 Intervention studies with high carbohydrate diet

Study	Subjects	Dietary change	Effects	Comments
Jenkins *et al.* 1987	6 healthy free living men	Cross-over design 2×2 weeks dietary intervention trial with high & low GI diet with 29 unit reduction in GI,	Significant reduction on low GI diet in serum fructoseamine (7%), TC (15%), 24-h urinary C-peptide level (32±10%).	Poor methodology & small sample size to assess lipid profile. Effects of other aspects of diet on TAG level and HDL-C level ignored. Consumed diets were not completely prepacked High GI diet also had a significant lower DF & unbalanced fibre content compared to low GI diet.
Wolever *et al.* 1992	15 type 2 diabetic patients,	Crossover design 2×2 weeks dietary intervention trial ,	Fasting serum fructosamine & cholesterol levels were significantly lower on low GI diet	Insulin related parameters such as IR not reported, in spite of the main problem in insulin level.
Jeppesen *et al.* 1997	10 healthy postmenopausal women followed for 3 weeks. Ratio of sugar to starch was identical (0.33 : 0.66)	Two isoenergic diet (15% protein, 60% CHO & 25% fat or 15% protein, 40% CHO & 45% fat	Significant decrease in insulin, HDL-C & significant increase in TAG, VLDL-TAG & VLDL-C after 60% diet.	No data on type (GI) of the CHO & GL of the consumed diet.
Luscombe *et al.* 1999	14 male & 7 female type 2 diabetics (obese)	Crossover design 2×4 weeks dietary intervention trial with high & low GI diet & 20 unit reduction in GI, dietary fibre was > than 30g on each diet.	HDL-C was higher on low GI but no significant difference in other metabolic parameters,	Some subjects suffered from uncontrolled diabetes and high BMI which directly affect carbohydrate metabolism. No report about the probable effects of diets on insulin sensitivity about 20-unit reduction in GI.
Heilbronn *et al.* 2002	23 female & 22 male overweight free living subjects with type 2 diabetes	Parallel study for 8 weeks with 32 unit reduction in GI	There was significant weight loss by the low or high GI diet	Low energy intake during interventions that affected the nature of diets & results. Instead of parallel design, subjects should undergo crossover design & insulin related parameters should be evaluated.
Brynes *et al.* 2003	17 males with risk factors. Assessment of lipid parameters such as HDL-C & insulin related parameters IR.	Intervention dietary trial over short and medium term with high and low GI diets. Postprandial sampling.	Benefical effects of high fat diet on postprandial IR but associated with increase in TAG & NEFA. High GI diet caused an increase in HOMA	Dietary advice rather than introduction of well defined & controlled diet. Subjects with low GI diet produced significantly lower HDL-C.
Frost *et al.* 2004	Male & female (30-70 years), free-living CHD patients, in a randomised parallel group trial over 12 weeks	Currently advocated healthy eating dietary advice compared with healthy eating advice emphasising LGI CHO	Low GI had a higher dietary fibre intake but there was no significant effect of two diets on lipid profile, insulin related parameters & anthropometric parameters.	Only nutritional & dietary advice given which may not have been complied with. Patients suffered from a life threatening disease so compliance with diet could be questioned.

1.8.2.3 Inflammatory markers

C-reactive protein (CRP) belongs to the Pentraxin protein family. It consists of 5 identical subunits of 206 amino acid residues. Five non-covalently associated protomers are arranged symmetrically around a central pore determined by X-ray crystallography (Shrive *et al.* 1996).

CRP is a plasma protein that is an essential part of the inflammatory process. It rises rapidly in serum in response to inflammatory stimuli (Volanakis and Kaplan, 1971; Volanakis, 2001). CRP is regulated by proinflammatory cytokines including interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) and biosynthesised in the liver (Hardardottir *et al.* 1994; Baumann and Gauldie, 1994). The main biologic function of this molecule is the recognition of pathogens, damaged cells and their elimination (Volanakis, 2001). Heinrich showed that in liver, the synthesis of CRP is largely regulated by IL-6 (Heinrich *et al.* 1990). In turn, it originates largely from activated leukocytes in the vessel wall (Danesh *et al.* 1997). CRP, along with other elements of the immune system, not only has protective characteristics but also may have harmful effects including atherogenic effects (Ridker *et al.* 2002). The mechanism underlying the relationship between inflammatory processes and CHD is poorly understood. However, a number of studies including the longitudinal Physician Health Study in the USA (Ridker *et al.* 1997; Ridker *et al.* 2002) have indicated that higher levels of plasma CRP and IL-6 are associated with higher CHD risk (Mendall *et al.* 1997; Yudkin *et al.* 1999; Ridker *et al.* 2002).

The lesions developed in atherosclerosis represent cellular responses to inflammatory diseases (Ross, 1999). Increased plasma CRP, a marker of systemic inflammation, is suggested to be a stronger risk marker of cardiovascular disease and sudden death than LDL cholesterol concentration confirmed by multivariable analysis after adjusting for common cardiovascular risk factors (Ridker *et al.* 1997). CRP levels have been reported to predict cardiovascular events and CHD mortality (Kuller *et al.* 1996; Koenig *et al.* 1999).

CRP also contributes to the insulin response as it is modulated by insulin (Campos and Bauman, 1992). This cytokine inhibits insulin signalling (Hotamisligil and Spiegelman, 1994). Insulin sensitivity was inversely associated with plasma CRP levels and total body fat (Sites *et al.* 2002). In addition, treatment with insulin accompanied by diet restriction among type 2 diabetic patients resulted in lower plasma CRP concentration (Yudkin *et al.* 1999).

The results of the Women's Health Study showed that plasma CRP concentration varied from 1.9 mg/L to 3.7 mg/L in the lowest up to the highest quintile. Those with the highest GL value had 9.4 times greater risk of having a higher value of plasma CRP. Dietary GL was positively and significantly correlated with CRP. The possible mechanism for the effect of high GL on CRP could be through stimulation of IL-6 secretion (Liu *et al.* 2002). IL-6 is as another inflammatory factor and modulator of fat metabolism in human and may predict CHD (Choi *et al.* 2004). IL-6 increases lipolysis and fat oxidation without causing hypertriacylglycerolaemia (van Hall *et al.* 2003) and enhancing endogenous glucose production (Pedersen *et al.* 2004). Although combined healthy eating recommendations (i.e. high fibre and LF

diet) and exercise have been shown to lead to significant reductions in plasma CRP level (Wegge *et al.* 2004), the direct effect of a change in dietary macronutrients or glycaemic index on CRP is not clear (Clifton, 2003).

1.8.2.4 Plasma antioxidant activity

Although there may not be much evidence for the benefits of a high carbohydrate diet, one consequence of such dietary advice is an increase in the intake of fruit and vegetables. Most of the UK population do not ingest the recommended 5 portions of fruit and vegetables a day.

One of the main benefits of increasing fruit and vegetable intake in relation to CHD is the increased intake of dietary antioxidants, which protect against free radical damage to plasma lipids increasing the resistance of low-density lipoprotein (LDL) to oxidation (Diaz *et al.* 1997, Luo *et al.* 1994) that facilates the process of atherosclerosis (Fig 1.17).

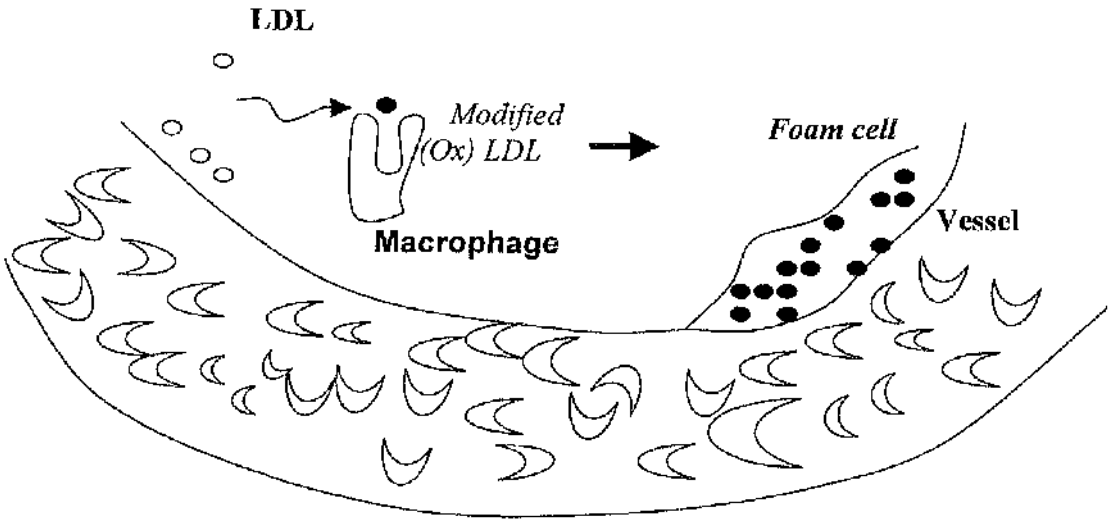
Free radicals are any species capable of independent existence that contain one or more unpaired electron. They are produced during the normal metabolic pathways in the cell and particularly in the mitochondria. These very reactive and unstable molecules include the super oxide anion (O_2-), hydroxyl ($OH^{\cdot}$), peroxyl ($LOO^{\cdot}$), hyperchlorous acid (HOCl), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O2$). Radicals such as nitric oxide (NO) or peroxynitrite ($ONOO^{\cdot}$) are nitrogen containing reactive molecules.

In order to become more stable, free radicals attempt to take an electron from a stable molecule and in doing so, they cause damage to PUFAs, protein and DNA. The body normally protects itself from these free radicals by adding another electron to the free radical or removing the unpaired electron. This is achieved by enzymes such as super oxide dismutase, catalase and glutathione peroxidase.

In addition dietary antioxidants such as water-soluble Vitamin C, pyridoxal phosphate, flavonoids and lipid soluble antioxidants such as Vitamin E and caretenoids work together to protect membrane lipids, protein and DNA. Vitamin C works in the cytoplasm but also regenerates Vitamin E which is situated in the membranes and protects PUFAs by donating a H from OH groups on its ring structure to a free radical, by donating a H. α-tocopherol becomes an unreactive free radical as the unpaired electron can be delocalized into the aromatic ring structure increasing its stability (Duvall, 2005).

Oxidation of LDL is critical to the formation of atherogenic plaques in blood vessels. Goldstein *et al.* (1979) showed that chemically modified (oxidised) LDL was taken up by macrophages resulting in the formation of foam cells which accumulated in atherosclerotic lesions (Itabe, 2003). This work led to them being awarded the Nobel prize.

Figure 1.1.6 Schematic processes for the formation of foam cell in vessel wall and contribution of macrophages.



Excessive fullness of macrophages with modified LDL cholesterol is the main characteristic of atherosclerotic plaques that cause most heart attacks and strokes (Kruth *et al.* 2002; Itabe, 2003).

Epidemiological studies strongly suggest that diets rich in fruit and vegetables, containing antioxidants such as vitamins C and E, carotenoids and flavonoids, protect against CHD (Leenen, 2001) and most evidence indicates that diets rich in antioxidants may reduce the risk of CHD by protecting against the adverse effects of free radicals (Ness *et al.* 1996). However it is not certain that advice to increase fruit and vegetable intake results in an increase in the antioxidant potential of plasma. This possibility is explored in one of the study described in chapter 3 of this thesis. Moreover, to date large scale randomized control trials of antioxidant vitamin supplements have failed to show a protective effect on CVD (Asplund *et al* 2002).

1.9 Rationale for the thesis

Carbohydrates are not only a main source of energy for the body but also interact with other macronutients (i.e. lipids and proteins) in human metabolism. The structure of dietary carbohydrates plays an important role in their digestion and absorption and glycaemic response. It is important that the quality and quantity of carbohydrates in the diet and their role in promoting health and and reducing risk of cardiovascular disease are studied. There is evidence indicating that the use of dietary GI (quantitative indicator) and GL (qualitative and quantitative indicator) is more advantageous than the traditional classification of dietary carbohydrates for management of a number of conditions such as type 2 diabetes. Results of large-scale population studies have revealed the probable association between dietary GI and GL with a number of chronic diseases as well as metabolic risk factors for CHD (e.g. plasma HDL cholesterol and TAG concentrations).

The current dietary recommendation is to provide at 50% (45%-65%) of dietary energy from carbohydrates, although results of the National Diet and Nutrition Survey indicates that less than half of energy intake is derived from carbohydrates in the UK. However, several short-term studies have shown possible adverse effects of a high carbohydrate diet on plasma HDL cholesterol and other risk factors for CHD. It was therefore decided to investigate the effect of these dietary guidelines on the eating habits and CHD risk factors of freeliving postmenopausal women. It is important to check that these dietary guidelines are appropriate.

Following on from this, it was decided to check if there was any relationship between GI and the GL of the diet of offspring of patients with type 2 diabetes and

matched controls, and their risk factors for CHD. Finally the relationship between GI and GL on these risk factors was studied in a short term intervention study in healthy adults.

These studies should show if the level of carbohydrate in the diet is detrimental to CHD risk factors in a range of individuals and whether the glycaemic response is more important than the amount of carbohydrate eaten.

1.10 Aim of the thesis

The aim of the thesis was to test the hypotheses

- A high carbohydrate diet increases CHD risk factors under freeliving conditions in post menopausal women
- GI and GL are more important factors in determining CHD risk factors than a simple measure of the amount of carbohydrate in the diet.

The objectives of the present thesis were

- To investigate the effect of advice to increase carbohydrate intakes as part of dietary advice to follow the UK dietary guidelines on some metabolic risk factors for CHD in healthy free-living postmenopausal women,
- To assess habitual dietary intake, GI, GL and the metabolic risk factors for CHD and type 2 diabetes,
- To examine the relationships between habitual dietary intake, GI, GL and metabolic risk factors in offspring of patients with type 2 diabetes and in control subjects,
- To examine the influence of high carbohydrate, isocaloric, high and low GI and GL diets for three days on metabolic parameters in the fasted state including plasma lipids, glucose, insulin, NEFA and inflammatory markers in healthy male subjects.

Chapter 2

Methodology

2.1 Introduction

This chapter details the methods of the three different studies that make up this thesis. Each study had its own design and subjects but many of the methods including dietary assessment, biochemical and anthropometric measurements were similar. The common methods are described in this chapter and experimental details specific to the different studies are included in the relevant chapters.

2.2 Summary of study protocols

2.2.1 First study: *Effect of increased carbohydrate intake (as part of advice to follow the UK dietary guidelines) on metabolic risk factors for CHD in healthy postmenopausal women*

This was a dietary intervention trial in which healthy postmenopausal women were given detailed dietary advice on how to change their habitual diet to increase carbohydrate intake in line with the current dietary guidelines. A fasting blood sample was taken at screening, baseline, and after 1 and 4 weeks of the dietary intervention to measure a number of metabolic parameters.

2.2.1.1 Objectives of the study

- To investigate the effect of advice to increase carbohydrate intake as part of the UK dietary guidelines on metabolic risk factors for CHD in healthy, free-living postmenopausal women.

2.2.2 Second study: *Relationships between dietary glycaemic index and metabolic parameters in offspring of patients with type 2 diabetes and control subjects*

A case-control study was carried out on thirty-four healthy subjects, 17 offspring of patients with type 2 diabetes mellitus (4 men and 13 women) and 17 age and sex-matched control subjects. Control subjects had no history of diabetes mellitus in the family.

2.2.2.1 Objectives of the study

The objectives of the study were to:

- Assess habitual dietary intake, glycaemic index (GI), glycaemic load (GL) and some common metabolic risk factors for CHD and type 2 diabetes.
- Examine relationships between habitual dietary intake, GI, GL and metabolic risk factors in offspring of patients with type 2 diabetes and in control subjects.

2.2.3 Third study: *Effect of high carbohydrate, isocaloric high and low glycaemic index diets on fasting plasma metabolic parameters in healthy male subjects*

A randomised-crossover dietary intervention trial of high carbohydrate, isocaloric high and low GI diets was conducted on healthy male volunteers. The duration of the intervention was three days on each trial with at least a two week washout period between trials.

2.2.3.1 Objectives of study

- To examine the influence of high carbohydrate, isocaloric, high and low GI diets for three days in healthy male subjects on a number of metabolic parameters.

2.3 Measurements

2.3.1 Anthropometric Measurements

All anthropometric measurements including height (m), weight (kg), waist circumference, hip circumference (cm) and mid-upper arm circumference (cm) (MUAC) were conducted in the fasted state. Height was measured using a stadiometer (Holtain Ltd, Crymych, Dyfed UK). The subjects' body weight was measured (SECA scales, Germany) in light clothes and no shoes. Body mass index (BMI) was calculated from weight and height (weight (kg)/ height (m^2)). Waist circumference was evaluated as an indicator of central obesity (Lean *et al.* 1995; Gray *et al.* 2000). It was measured midway between the lower margin of the last rib and the crest of the ilium, in the horizontal plane using a non stretch tape without compressing any soft tissues to the nearest 0.1 cm. Hip circumference was measured at the top of iliac crest using a tape measure to the nearest 0.1 cm (Garrow *et al.* 2000).

Body fat was estimated by skinfold thickness measurements. The skinfold measurement was made using calipers (Holtain Ltd. Crymych, UK), which measure the thickness of a fold of skin with its underlying layer of fat. Skinfold thickness was measured at four different sites; biceps; triceps; subscapular, and suprailliac. Subcutaneous fat is not uniformly distributed on the body. Thus, taking skinfold

measurement at different sites on the body (such as triceps, biceps, subscapular and suprailiac) will help to account for the differing distribution of subcutaneous fat. Three measurements were taken at each site and averaged. Then, the sum of the four values was used to calculate the percent of body fat for each subject using the equations of (Durnin and Womersley, 1974).

2.3.2 Dietary assessment

Dietary assessment is an essential tool for studying relationships between dietary exposure and disease causation. A number of methods are available to assess individual dietary intake. None will provide a truly accurate picture of the habitual dietary intake of an individual because of the complex nature of diet (Willett, 1998). There is no ideal or gold standard method and therefore, the choice of method depends on the design and objectives of the study (Nelson and Bingham, 1997).

Dietary assessment methods can be classified as prospective or retrospective techniques that assesses food intake in the present or past (Nelson and Bingham, 1997). Retrospective methods include 24-hr diet recall, food frequency questionnaire (FFQ) and the diet history method (Bingham *et al.* 1994) Prospective methods include keeping a food diary and either weighing actual foods consumed or estimating the weights of the foods. A 24-hr diet recall is an interview in which respondants describe all of the foodstuffs they consumed in past 24 hours. A FFQ is questionnaire containing a list of foods with questions on the frequency of consumption, such as per day, per week, per month or rarely. Subjects can complete a FFQ themselves or the questionnaire can be administered by an interviewer (Schatzkin *et al.* 2003). FFQ tend to be used in large-scale epidemiological studies

where the researchers want to rank individuals by dietary intake or compare dietary intake of specific nutrients by group.

A diet history is an interview by a trained person who tries to build up a picture of a typical pattern of food intake during a recent week. Food intake is usually quantified either by using household measures or food photographs. There are advantages and disadvantages of both prospective and retrospective methods. The advantages of retrospective methods are that they do not require a lot of effort from the subjects. Another advantage of retrospective methods, especially the FFQ, is that these questionnaires can in some cases be coded by machine and this substantially reduces the cost involved of analysing this data. The main disadvantage of retrospective methods is that they are reliant on the respondent's memory. Difficulty in accurately remembering previously consumed foods and beverages is the main weakness of these methods. Also, accurate memory of past diet can be influenced by the subject's current eating habits (Bingham *et al.* 1994).

Prospective methods that involve keeping records of foods and beverages consumed at the time of eating were for a long time considered to be the gold standard of dietary assessment. These methods involve the subject recording everything that they eat and drink during a specified period of time, usually between 1-7 days (Gibney *et al.* 2002). Subjects should be given clear and detailed instructions on how to do this and this will minimise the errors involved. These methods, if carried out properly, can provide highly detailed information on foods consumed. Sometimes subjects are asked to weigh foods before eating, while in other studies subjects are asked to quantify the amount of foods consumed by household measures or photographs.

The main advantage of these methods as a way of assessing diet is that they are in general more reliable and precise for estimating mean intake of nutrients as they are less influenced by the subjects' memory (Lee-Han *et al.* 1989). As regards the length of time for which subjects need to record their food intake, it has been suggested (Gibson, 1993) that a minimum of two week days and one weekend day should be included to take account of potential differences in food intake patterns. However, (Bingham *et al.* 1994) have recommended that 7 days are needed to accurately assess macronutrient intake.

There are disadvantages of food records as a dietary assessment method, including the fact that they require a high level of respondent literacy and subjects need to be able to read and write i.e. therefore not suitable for using in a study on young children. Another disadvantage is that subjects need to to be very well motivated as keeping food records for a number of days is quite a lot of work and effort. Carrying out a weighed food record can be very inconvenient for the subject, especially for meals that are consumed outside of the home, ie. restaurant or party. Furthermore these methods place a great burden on the respondents and are very time consuming. The food records are expensive to analyse, and therefore their use tends to lead to higher dropout rates. For these reasons, food records tend not to be used in large-scale studies (National Institute of Health Guide, 1992; Bingham *et al.* 1994). Also, it well known that underreporting is a common problem associated with these methods (Bingham *et al.* 1994).

In the studies in this thesis, weighed food records were the chosen method for dietary assessment. This method was also used to assess compliance to dietary interventions. Subjects were asked to keep a record of all food and drink consumed. Each food item

was weighed immediately prior to consumption using a portable battery operated food weighing scales (Slater Household Ltd, Tonbridge, UK). In the first study, postmenopausal women were asked to keep a 7-day weighed food record for the assessment of habitual diet and a 3-day weighed food record for each week over the 4-week dietary intervention to assess compliance to the dietary advice. In the second study, subjects carried out a 7-day weighed food record to assess their habitual diet. In the third study, volunteers carried out a 3-day weighed food record to assess their habitual diet and they also weighed their food consumption according to the amounts specified on their individualised menu plan. They were also asked to note changes, if any, in their food consumption on the menuplan. This was then anslysed for complance to the intervention diets.

The information recorded in the food diaries was imputed and analysed using a computerised version (Diet 5^{TM}, Robert Gordon University, Aberdeen) of the food composition tables (Holland *et al.* 1991). The dietary records were analysed for energy intake, absolute amounts of carbohydrate, fat, protein, alcohol and a some vitamin and minerals and also for the proportion of energy from the main energy providing nutrients.

Biomarkers are a very important part of dietary assessment because they are objective, which means that do not have the errors and bias that are associated with self-reporting diet. An example of a useful biomarker for assessing energy intake is the use of doubly labelled water to estimate energy expenditure. Also, 24-h urinary sodium excretion is reported to be a reliable measure of sodium intake. Serum carotenoid concentrations could be used to confirm if subjects followed advice to increase their fruit and vegeable intake in an intervention study. However, there are

some nutrients for which there are no biomarkers currently available, e.g. total fat intake. Obviously using biomarkers in dietary studies adds to the cost of doing a study and depending on the biomarker needed may not be feasible to use in some studies. Another consideration is that body tissues may be needed (e.g. blood or urine) and depending on the resources available, it may not be feasible to carry out this biomarker analysis. In spite of these problems associated with the use of biomarkers, there is increasing awareness of the importance of using biomarkers to help with the interpretation of results in studies that rely on self-reported diet (Gibney *et al.* 2002).

Studies which have used doubly labelled water as a biomarker for energy intake have highlighted the problem of under-reporting of dietary intake by showing that some reported energy intakes were biologically implausible. It also seems to be the case that certain people are more likely to underestimate their dietary intake, and in particular women, obese indivuals, people who are conscious about their weight, and people who are not very motivated to do the study in the first place (Samaras *et al.* 1999). It has also been suggested that people under-estimate or under-report some foods such as fat-rich foods or desserts more than other foods. However, it is possible to eliminate dietary intake estimates that fall below a certain cut-off point of biological plausibility. For example, energy intakes estimated by FFQ are usually not used if the energy intake values are above 5,000 kcal or below 1,000 kcal as it assumed that these FFQ had not been completed properly (Date *et al.* 2005). If measures of weight are available, it is possible to estimate basal metabolic rate (BMR) using the Schofield equations (Schofield *et al.* 1985) and this allows investigators to identify and eliminate under- or over-reported data. In this thesis, the Schofield equations (Schofield *et al.* 1985) were used to estimate BMR, and

subjects' diet records were not included in the statistical analysis if their reported energy intakes were less than their estimated BMR multiplied by 1.1 (under-reporters) or greater than 2.0 (over-reporters). These cut-offs are used as it is highly unlikely that habitual energy intake would be < 1.1 × BMR or > 2.0 × BMR (Black *et al.* 1991; Goldberg *et al.* 1991). However, it must be admitted that the use of these cut-offs are limited and imperfect as they only exclude individuals who have reported biologically implausible energy intakes and do not identify individuals who mis-report to a lesser degree.

2.3.3 GI and GL calculation

In each study, we estimated the GI and GL of the diet. GI values were taken from the The International Table of GI and GL (Foster-Powell *et al.* 2002). The following steps were applied for each subject to determine the appropriate average GI and GL:

- A description of the foods that subjects consumed and the amount in grams were provided in the subjects' food records.
- The carbohydrate content of each food was determined using Diet 5™ software.
- The appropriate GI for each food was found in the International Table of GI and GL (Foster-Powell *et al.* 2002).
- If GI values for a specific food was not available in the table, where possible a value for a similar and appropriate food was used.

In general, the GI value for foods was an average from a number of studies involving healthy individuals as well as patients with type 1 and type 2 diabetes. The similarities in GI values derived in subjects using healthy individuals and patients

with diabetes has been discussed by Wolever *et al.* (1987). It has been shown that GI values determined in healthy subjects correlate well with those found for diabetic subjects (Wolever *et al.* 1987). Even though individuals with diabetes have higher blood glucose values, they are members of the same human species and they show the same differences in rates of digestion and absorption of carbohydrates.

The International Table of GI and GL contains relevant GI values data published from 1981 to 2001 and contains nearly 1300 separate entries (more than 750 different types of foods) (Foster-Powell *et al.* 2002).

(Laville 2004) suggested that differences in testing methods such as different types of blood sampling (venous or capillary), the variation for the same food due to inherent botanical differences from country to country, differences in amylose to amylopectin ratio, type of cooking method, and differences in methods of food processing could explain the variations in GI observed for a single food item.

The glycaemic load of individual foods was calculated by multiplying the amount of carbohydrate, in grams in each food (obtained from Diet 5^{TM}, Robert Gordon University, Aberdeen) with their respective GI value, and dividing by 100. The GL for each diet was obtained by finding the sum of each individual GL value. The GI of each diet was calculated by dividing the GL of the diet by the total amount of carbohydrate in the diet (obtained from Diet 5^{TM}) then multiplying by 100.

2.4 Outcome measures

2.4.1 Laboratory analysis

2.4.1.1 Fasting blood samples

Subjects were asked to refrain from alcohol and vigorous physical activity for 24h before visit and fasting blood samples were collected by an experienced person (after a 12 hr over night fast) into vacutainers containing ethylenediamintetra-acetic acid (EDTA), lithium heparin or fluoride oxalate.

2.4.1.2 Oral glucose tolerance test (OGTT)

In order to assess the insulin sensitivity and insulin resistance indices in the second study, subjects had an OGTT. They arrived at the laboratory after an overnight fast (12 h). At the beginning of the test, each subject had a cannula that inserted in the ante-cubital fossa vein in the non-dominant arm. Blood samples were taken before and 15, 30, 60, 90, and 120 minutes after subjects consumed a 75g oral glucose load (75g glucose dissolved in 250 ml water). Sterile saline (9g/l NaCl, 5 ml) was used to prevent blood from clotting in the cannula throughout the test period.

Blood samples were kept on ice, and plasma was separated as soon as possible and idealy within 10 minutes of collection. The blood samples were centrifuged at 3500 revolutions per minute (rpm) for 10 minutes at 4°C using a clinical centrifuge (Mistral 3000i, Sanyo Gallenkamp plc, Leicester, UK). Plasma was divided into aliquots and stored at –70° C until analysis.

Plasma samples, collected in EDTA, were used for triacylglycerol (TAG), total and HDL cholesterol, C-reactive protein (CRP), non-esterified fatty acids (NEFA) and

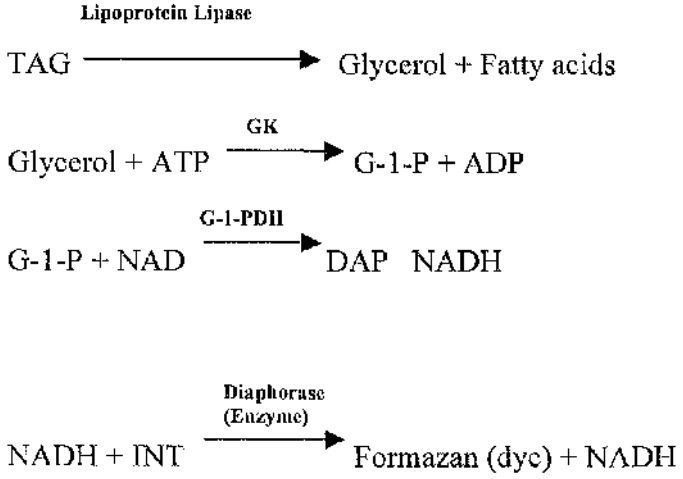
adiponectin analysis and plasma collected in lithium heparin was used for insulin determination. In the postmenopausal women study (study 1) and the diabetic relatives study (study 2), plasma collected in fluoride oxalate was used for glucose, but in the intervention study (study 3) glucose was analysed from EDTA plasma.

2.4.2 Lipid parameters

Lipid parameters in the first study were assessed using kits and the methods are briefly described below. The lipid measurements related to the second and third study were measured in the Department of Pathological Biochemistry at the University of Glasgow. Fasting plasma lipids including total and HDL cholesterol, and triacylglycerol concentrations were determined using an automated Hitatchi 197 multichannel analyser (Roche Diagnostics, Lewes, East Sussex, UK) using standard procedures. LDL cholesterol concentrations were calculated using the Friedewald formula (Friedewald *et al.* 1972).

2.4.2.1 Triacylglycerol (TAG)

Methods for assessing TAG usually involve enzymatic or alkaline hydrolysis to glycerol and fatty acids followed by the chemical or enzymatic measurement of the glycerol component. The kit that was used involved the hydrolysis of TAG by the enzyme lipase to glycerol and free fatty acids. The glycerol produced was coupled by enzyme reactions catalyzed by glycerol kinase (GK), glycerol-1-phosphate dehydroenase (G-1-PDH) and diaphorase. The formazan produced is highly coloured and has an absorbance maximum at 500 nm. The intensity of the color produced is directly proportional to the TAG concentration of the sample. The enzymatic reactions involved in the assay are as follows:

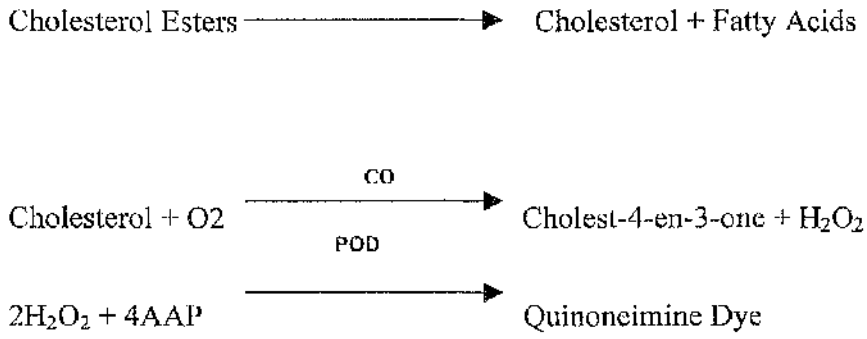


The procedure (Sigma, procedure no. 336) briefly involved mixing 1 ml of triglyceride reagent (provided in the kit) to 0.01 ml of sample or standard, which are then incubated at 15 min at 37°C. The absorbance of the samples and standard were then read using a spectrophotometer (Thermo Labsystems Multiscan, Vaanta, Finland) at 500 nm. A standard curve was constructed and the equation of the line was used to calculate the concentration of the TAG in the plasma samples in mg/L. TAG values were then converted to mmol/L by multiplying by the conversion factor of 0.0113. A coefficient of variation (CV) of 2.2% was obtained for TAG determination.

2.4.2.2 Total cholesterol

Methods for assessing total cholesterol usually involve enzymatic reactions followed by the chemical or enzymatic measurement of the developed and coloured adducts. The kit that was used involved the enzymatic hydrolysis of cholesterol esters by cholesterol esterase (CE) to free cholesterol and free fatty acids. Oxidation of free cholesterol by cholesterol oxidase (CO) to cholest-4-en-3-one and hydrogen peroxide and at the end, combination of hydroxylbenzoic acid (HBA) and 4-aminoantipyrine

(4AAP) in the presence of peroxidase (POD) to form a chromophore (quinoneimine dye) which has its λ_{max} is 500 nm are the major steps in the determination of plasma or plasma or serum cholesterol level. The enzymatic reactions involved in the assay are as follows:



The procedure (Sigma, procedure no.401) briefly involved mixing 1 ml of the reagent (provided in the kit) to 0.01 ml of sample or standard, which are then incubated for 15 min at 37°C. The absorbance of the samples and standard were then read using a spectrophotometer (Thermo Labsystems Multiscan, Vaanta, Finland) at 500 nm. A standard curve was constructed and the equation of the line was used to calculated the concentration of the cholesterol in the plasma samples in mg/L. Cholesterol values were then converted to mmol/L by multiplying by a conversion factor of 0.0259. A coefficient of variation (CV) of 1.6% was obtained for cholesterol determination using the Sigma kit in the first study.

2.4.2.3 HDL-cholesterol

The method for assessing HDL-cholesterol involves the precipitation and removal of apo-B containing lipoproteins, so that the apolipoproteins of HDL which are apo A-I and apo AII remain, and then the cholesterol component is measured. The kit that I used in my first study involved phosphotungstic acid (30.3 mmol/L) and magnesium chloride (100 mmol/L) (PTA/ Mg Cl_2) as active ingredients. HDL cholesterol was

assessed after precipitation of apolipoprotein B-containing lipoproteins with PTA/ Mg Cl_2 mixture by Sigma diagnostics kit No. 352-4. The precipitated lipoproteins are removed from tube by centrifuging at 500g for 10 min by micro-centrifuge (MSE micro-centrifuge, UK).

Then the cholesterol precipitated is measured using same kit for cholesterol that was already been described. The procedure (Sigma, procedure No. 352.4) briefly involved mixing 1 ml of ready to use reagent (provided in the kit) with 0.05 ml supernatant of the extracted plasma after precipation of the other lipoproteins of plasma samples (EDTA as anticoagulant reagent) and which are then incubated at 15 min at 37°C. The absorbance of the samples and standard were then read using a spectrophotometer (Thermo Labsystems Multiscan, Vaanta, Finland) at 500 nm. A standard curve was constructed and the equation of the line was used to calculate the concentration of the HDL-cholesterol in the plasma samples in mg/L. Cholesterol values were then converted to mmol/L by multiplying by a conversion factor of 0.0259. A coefficient of variation (CV) of 2.8% was obtained for HDL-cholesterol determination using the Sigma kit in the first study.

2.4.2.4 Low-density lipoprotein (LDL) cholesterol

Low-density lipoprotein (LDL) cholesterol concentration was estimated by the Friedewald equation (Friedewald et al. 1972) using the following equation:

LDL-cholesterol(mg/dl) = total cholesterol - (HDLcholesterol + triacylglyceride/5).

2.4.3 Non-essential fatty acids (NEFA)

NEFA was assessed in plasma samples using an *in vitro* enzymatic colorimetric method (Wako Diagnostics Richmond Virginia, USA). This assessment was done at the department of Pathological Biochemistry at Glasgow Royal Infirmary using an automated Hitatchi 197 multichannel analyser. The Wako enzymatic method relies upon the acylation of coenzyme A (CoA) by the NEFA catalysed by acyl-CoA synthetase. The acyl-CoA thus producted is oxidized by added acyl-CoA oxidase with generation of hydrogen peroxide. Hydrogen peroxide in the presence of peroxidase permits the oxidative condensation of 3-metyhl-N-(β-hydroxyethyl)-aniline with 4-amino antipyrine to form a purple colored adduct which can be measured colorimetrically at 550 nm. The formation of this purple colour is directly proportion to concentration of NEFA in plasma samples.

2.4.4 Glucose

Glucose concentrations in plasma samples for the first study were assessed using a commercial kit (Glucose GOD-PAP, BioStat Diagnostic Systems, Cheshire, UK). This ''GOD-PAP'' enzymatic colorimetric test involved the determination of glucose concentrations after enzymatic oxidation with glucose oxidase (GOD). The colorimetric indicator is quinoneinine, which is generated from 4-aminoantipyrine and phenol by hydrogen peroxide under the catalytic action of peroxidase (POD). The enzymatic reactions involved in the assay are as follows:

$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$2\,H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + 4\,H_2O$$

The procedure briefly involved mixing 1 ml of glucose reagent (provided in the kit) to 0.01 ml of sample or standard, which are then incubated for 10 min at 37°C. The absorbance of the samples and standard were then read against a blank (0.01 ml distilled water + 1 ml reagent) using a spectrophotometer (Thermo Labsystems Multiscan, Vaanta, Finland) at 500 nm. A standard curve was constructed and the equation of the line was used to calculate the concentration of the glucose in the plasma samples in mg/L. Glucose values were then converted to mmol/L by multiplying by the conversion factor of 0.0551. A coefficient of variation (CV) of 1.8% was obtained for this assay.

Plasma glucose concentrations for the second and third studies were determined at the Department of Pathological Biochemistry at Glasgow Royal Infirmary using standard procedures. Briefly, this method (Gluco-quant Glucose/HK, Roche Diagnostics Corporation, Lewes, UK) involved the phosphorylation of glucose with ATP by hexokinase to produce glucose-6-phosphate (G-6-P). The produced G-6-P, which then reacts with $NADP^+$ catalyzed by glucose-6-phosphate dehydrogenase (G-6-PDH) to produce gluconate-6-phosphate and NADPH. The formation of gluconate-6-phosphate during the reaction is directly proportional to the glucose concentration, which is measured photometrically using an automated Hitatchi 197 multichannel analyser.

2.4.5 Insulin

Insulin is a polypeptide hormone (MW 6000) composed of two joined non-identical chains A and B, which are joined by disulfide bonds. Insulin is formed from a

precursor, proinsulin in the beta cells of the pancreas. Insulin is stored in the secretory granules of the islet cells of the pancreas and is then secreted. Insulin is secreted in response to an increase in blood glucose concentration.

Insulin measurements for the studies described in this thesis were carried out by the Department of Biochemistry at Glasgow Royal Infirmary. Insulin was measured in plasma using the Abbot IMx Insulin Assay kit (Abbot Laboratories, Tokyo, Japan) which is a Microparticle Enzyme Immuno Assay (MEIA).

Briefly, to carry out the IMx Insulin assay, the reagents in the kit and samples are added to cells in the following order:

- The sample, the antibody (anti-insulin, Mouse, Monoclonal coated microparticles) and the assay buffer are added to the incubation wells, and a reaction takes place in which an antibody-insulin complex is formed.
- An aliquot of the reaction mixture containing insulin bound to the anti-insulin coated microparticles is transferred to the glass fiber matrix.
- The matrix is washed to remove unbound materials.
- An anti-insulin: alkaline phosphatase conjugate is dispensed onto the matrix and binds to the antibody-antigen complex.
- The matrix is washed to remove unbound materials.
- The substrate, 4-methylumbelliferyl phosphate, is added to the matrix and the fluorescent product is measured by the MEIA optical assembly.

The sensitivity of the IMx analyser insulin assay was calculated to be 1.0 μU. Results of a study on 148 normal fasting plasma showed a mean ± 3SD

concentration equal to 7.1± 15.6 μU/ml. The reference range for fasting subjects was <13 $\mu U.L^{-1}$ and there was a mean interassay imprecision (CV) of 5.0% across the range 4-120 $\mu U.L^{-1}$.

2.4.6 C-reactive protein (CRP)

CRP is defined as a marker of systemic inflammation (Volanakis, 2001). It has recently gained more reognition as a predictor of cardiovascular disease (CVD) (Clifton, 2003). The results of the Physicians Health Study (Ridker *et al.* 1997; Ridker *et al.* 2002) in the USA revealed that higher levels of CRP and IL-6 were associated with higher CHD risk (Mendall *et al.* 1997; Yudkin *et al.* 1999; Ridker *et al.* 2002). CRP level has been reported to predict CVD events and CHD mortality over 2 and 17 year follow up in the MONICA-Ausburg cohort (Multinational MONItoring trends and determinants of CArdiovascular diseases) study (Koenig *et al.* 1998) and the MRFIT study (Kuller *et al.* 1996), respectively. It is also suggested that CRP is a stronger predictor of CVD than LDL cholesterol concentration after adjusting for a number of risk factors (Ridker *et al.* 1999; Ridker *et al.* 2002; Guerrero-Romero and Rodriguez-Moran, 2003; Ridker *et al.* 2003b). The advantage of CRP compared to the other inflammatory markers is that the relationship with CHD risk for inflammatory factors such as interleukin-6 was not as strong after controlling for common risk factors, while CRP remained a significant predictor even after controlling for these confounding factors (Ridker *et al.* 2002).

There are no intervention studies that have investigated the direct effect of glycaemic index (GI) or glycaemic load (GL) on CRP or any other inflammatory markers (Clifton, 2003). In the first and third studies in this thesis, subjects increased their

carbohydrate intake and their dietary GI and GL were altered and this provided an opportunity to investigate this issue. We could only find one published study (Liu *et al.* 2002) which was an analysis on a sub sample of the Women's Health Study Cohort and reported a strong statistically significant positive association between GL and CRP levels, with the odds ratio for the highest GL quintile compared to the lowest being 9.43 (95% CI 1.92, 46.23), suggesting that diets with higher GL are associated with higher CRP levels.

The Department of Pathological Biochemistry laboratory at Glasgow Royal Infirmary carried out the assessment of CRP using an in house ELISA (Highton & Hessian, 1984). CRP was measured using a highly sensitive double antibody sandwich ELIZA with rabbit anti-human CRP and peroxide conjugated rabbit anti-human CRP. The substrate for the colour development was 1,2 phenylaminediamine and the standard used was human CRP calibrator.

Median CRP concentrations for subjects who appear clinically well are in the region of 0.7- 0.8 mg/l with 90% having values of < 3.0 mg/l.

2.4.7 Interleukin-6 (IL-6)

IL-6 is another marker of inflammation, which is associated like CRP with increased risk of CHD (Ridker *et al.* 2000; Pradhan *et al.* 2002) and type 2 diabetes (Hu *et al.* 2004). It's level in the circulation has been shown to be associated with insulin sensitivity, and this association is independent of BMI (Fernandez-Real *et al.* 2001). Elevation of glucose levels has been shown to significantly increase IL-6 and other

inflammatory cytokines (IL-18, TNF-a) in both normal and impaired glucose tolerant (IGT) subjects (Esposito *et al.* 2002).

In my third study, IL-6 was determined using a quantitative sandwich ELIZA kit (R&D Systems, Europe Ltd). The Quantikine High Sensitivity kit was chosen as all of the subjects were healthy and no extreme values were expected. IL-6 was measured by a trained technician at the Department of Human Nutrition. This ELISA was designed to measure human IL-6 in serum, plasma and urine. A monoclonal antibody specific for IL-6 is precoated to the wells. Standards and samples are pipetted into the wells and the immobilized antibody binds IL-6. After incubation period of two hours any unbound substances are washed away and an enzyme-linked polyclonal antibody specific for IL-6 added to the wells. After another incubation period of two hours any unbound antibody-enzyme reagent is washed and a substrate solution is added to the wells. Another incubation period of 60 minutes at room temperature follows and after that an amplifier solution is added to the wells and colour develops in proportion to the amount of IL-6 bound in the first step of the assay. Within 30 minutes, a stop solution is added to each well and colour development stops. Finally the samples are read at 490 nm with a wavelength correction set to 650nm or 690nm using a plate reader (Thermo Labsystems Multiscan, Vaanta, Finland). A standard curve is constructed using the values of the standard solutions while the mean value of the duplicate samples is used to determine IL-6 concentration in $pg.ml^{-1}$. The R-squared of the reference curve for this assay was 0.9996 while the mean CV of the duplicates was 4.1%.

2.4.8 Adiponectin

Adiponectin is a plasma protein secreted specifically by adipose tissue (Maeda *et al.* 1996). Higher levels of adiponectin in the body are associated with improved insulin sensitivity, reduced inflammation and better glycaemic control. Although there are very few studies that have evaluated wheter diet has any effect on plasma adiponectin concentrations, Qi *et al.* (2005) studied this in male subjects with type 2 diabetes. Results of this cross-sectional study among 780 diabetic men from the Health Professionals' Follow-up Study showed that after adjustment for age, BMI, smoking and a number of other risk factors for CHD, dietary GI and GL were inversely associated with plasma adiponectin concentrations. P for trend was 0.005 for GI and 0.004 for GL. Circulating levels of adiponectin were 13% lower in the highest quintile of GI than its level in the lowest quintile. Assessment of dietary GL showed the similar trend and adiponectin levels were 18% lower in the highest quintile of GL than in the lowest GL. Furthermore dietary fibre intake was positively associated with the circulating level of adiponectin.

In the second study (chapter 4), adiponectin concentration was measured in EDTA plasma using an ELISA at the Pathological Biochemistry Department of Glasgow Royal Infirmary. Pre-treated samples and serially diluted standard (recombinant human adiponectin) solutions were added to an appropriate number of wells of the microtiter plate and incubated at room temperature for 60 mins. After washing, the secondary rabbit anti-adiponectin antibody was added to each well and incubated at room temperature for 60 mins. After washing, a conjugate of horseradish peroxidase and goat anti-rabbit IgG was added to each well and again incubated at room temperature for 60 mins. After washing, the colorimetric substrate for the enzyme

was added to the wells and incubated at room temperature for 15 mins. The colour development was terminated by the addition of a stop solution. The intensity of the colour was measured at 450nm on a VERSAmax Tm plate reader. The concentration was calculated using the absorbance values of the adiponectin standard solutions assayed at the same time. Results were quoted as $mg.l^{-1}$. The CV value was 3.5% for this assay.

2.4.9 Ferric reducing ability of plasma (FRAP) assay

Antioxidants are compounds that can protect the body or foods against the potentially harmful effects of free radicals or reactive oxygen species (ROS) (Halliwell and Gutteridge, 1995). The human body is constantly under attack from free radicals and ROS which are produced endogenously in the body as by products of normal aerobic metabolism and enzyme systems as well as being supplied from sources outside the body such as cigarette smoke, lipid peroxidation products in foods or pollutants. In order to protect the body from inappropriate exposure to these free radicals and ROS, the human body has developed a powerful and complex antioxidant defence system. Enzymatic antioxidants within the cell, such as superoxide dismutase function by inactivating or removing free radicals and ROS from the cell before they can cause damage. Non-enzymatic protein antioxidants function by controlling the storage and release of metal ions which are needed for the enzymatic antioxidants to function but can also convert relatively unreactive radicals such as superoxide to the much more reactive hydroxyl radical. However, antioxidants such as vitamin C, α-tocopherol, carotenoids and phenolic compounds such as flavonols act by donating electrons to free radicals in order to stabilise them and in this way behave as reducing agents. It is this ability to donate an electron or to

act as a reducing agent that is used to measure 'antioxidant potential' in the FRAP assay.

The FRAP assay offers a simple index of 'antioxidant or reducing power', and the results are reported to be highly reproducible (Benzie and Strain, 1996). However, there are also a number of limitations associated with the use of this assay for measuring 'antioxidant power'. The most obvious limitation of the FRAP assay is that while it claims to measure 'antioxidant power', it actually only measures 'antioxidant power' of non-enzymatic antioxidants that act as reducing agents. While the FRAP assay can be used to measure the 'antioxidant power' of foodstuffs, the results do not give us any information on the bioavailability of these antioxidants in the body and thus about their actual physiological 'antioxidant power' *in vivo*. In addition, substances that bind with either Fe^{3+} or Fe^{2+} could in theory interfere with the results of the assay (Benzie and Strain, 1996).

The method measures the ability of antioxidants in plasma or in foods to reduce the ferric component (Fe^{3+}) of a ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex (which is contained in the FRAP reagent) to the ferrous form (Fe^{2+}). During this reaction which takes place at a low pH, the reduction of ferric iron (Fe^{3+}) to the ferrous form (Fe^{2+}) is accompanied by the formation of a blue colour which can be measured at an absorption maximum of 593nm using a spectrophotometer (Benzie and Strain, 1996).

The FRAP assay was carried out according to the methods of Benzie and Strain, (1996). It is a simple spectrophotometric assay that assay involves making up a

FRAP reagent, making up the 1.0 mM ferrous sulphate ($FeSO_4$, Fe^{2+}) standard solution, then making dilutions of the stock standard to produce standards of range 0-1.0 mM FeSO4, and then carrying out the FRAP assay with the plasma samples.

The FRAP reagent (120 ml) was prepared freshly on each working day and should be orange-brown in colour. It was prepared by mixing the following solutions together:

- 100 ml acetate buffer (300 mM acetic acid, pH 3.6)
- 10 ml TPTZ solution (10 mM, made up with 40mM HCl)
- 10ml ferric chloride solution (20 mM, made up in distilled water)

A standard curve was prepared according to the following table from $FeSO_4$ (1 mM).

Table 2.1 Standard solutions of $FeSO_4$ (1mM) preparation

Final concentration of $FeSO_4$, (mM)	**Volume (ml) of 1 mM $FeSO_4$ to be added**	**Volume (ml) of distilled H_2O to be added**
0.0 (Blank)	0.0	5.0
0.2	1.0	4.0
0.4	2.0	3.0
0.6	3.0	2.0
0.8	4.0	1.0
1.0	5.0	0.0

The FRAP solution and distilled water were kept warm throughout the procedure by keeping in a waterbath at 37°C. As shown below, 100µl of each concentrations of

standard or plasma sample was pipetted into into a cuvette. 300µl of distilled water was then pipetted into the cuvette. Then, at 30-second intervals, 3ml freshly prepared warmed FRAP solution was then pipetted into the cuvette. Only six standards or samples were prepared at a time. The standards were analysed in triplicate and the plasma samples in duplicate. The contents of the cuvettes were then mixed by inverting (with lids on). The cuvettes were then incubated for exactly 4 min at 37°C in an incubator.

The absorbance of each standard, and plasma sample was then measured at 593nm using the plate reader. A standard curve was constructed from the concentrations of the standards and the absorbance values using Microsoft Excel, and the equation of the line was was found. The equation of the line was then used to calculated the FRAP concentration of the plasma samples (in mM $FeSO_4$). The CV for the analysis of the plasma samples was 2.7%.

2.4.10 Homeostatic Model Assessment ($HOMA_{IR}$) Score

There are a number of techniques available to measure insulin sensitivity such as the euglycemic clamp method, the insulin tolerance test (ITT), the oral glucose tolerance test (OGTT), the intravenous glucose tolerance test (IVGTT), the insulin suppression test (IST), and the homeostatic model assessment of insulin resistance ($HOMA_{IR}$). Each technique has advantages and disadvantages and various tests can be validated against each other. However, it has now been generally accepted that the euglycemic clamp technique is the 'gold standard' method for assessing insulin action *in vivo* (Isomaa, 2003). This method involves the infusion of glucose to provide a constant blood glucose concentration (euglycemia) in all subjects, and provides a scale to compare insulin sensitivity. The greater the quantity of glucose needed to produce

euglycaemia the greater sensitivity to insulin. Although this method is an important and precise procedure for assessing insulin sensitivity, it is a demanding, complicated and expensive technique involving intravenous insulin infusion (Scheen *et al.* 1994; Isomaa, 2003). Other methods used to assess insulin sensitivity are simpler, less costly and invasive compared with the euglycemic clamp technique and have been validated against this gold standard method.

Matthews *et al.* (1985) also presented another method, which is a calculation using fasting glucose and insulin, the haomostatic model assessment for assessing insulin resistance ($HOMA_{IR}$). The $HOMA_{IR}$ model was developed and validated against the hyperinsulinemic-euglycemic clamp (for insulin resistance) and the hyperglycemic clamp (for insulin secretion). In this thesis insulin resistance was estimated using the $HOMA_{IR}$ technique. $HOMA_{IR}$ was estimated as follows:

$HOMA_{IR}$ score = Fasting insulin (μU/ml) X fasting glucose (mmol/L) /22.5

2.4.11 Insulin-sensitivity index (*ISI*) and insulin-resistance index (*IRI*)

In the second study (Chapter 4) insulin sensitivity was assessed using the Belfiore *et al.* (2001) method. This method was used to determine the insulin sensitivity because of its feasibility and accuracy compared with the gold standard technique. This method for assessing insulin sensitivity uses fasting and oral glucose tolerance test (OGTT), glucose, insulin and NEFA concentrations, has been validated against the euglycemic clamp technique.

The insulin sensitivity index for glycemia ($ISI_{(gly)}$) and insulin sensitivity index for blood free fatty acids (FFA) ($ISI_{(ffa)}$) were calculated as follows (Belfiore *et al.* 2001):

$$ISI_{(gly)} = 2/[(Insulin_{AUC} \times Gly_{AUC}) + 1], \text{ and } IRI_{(gly)} = 2/[1/(Insulin_{AUC} \times Gly_{AUC})] + 1$$

$$ISI_{(ffa)} = 2/[(Insulin_{AUC} \times FFA_{AUC}) + 1], \text{ and } IRI_{(ffa)} = 2/[1/(Insulin_{AUC} \times FFA_{AUC})] + 1$$

$Insulin_{AUC}$, Gly_{AUC} and FFA_{AUC} indicate insulinemic, glycemic and blood free fatty acid areas under the curve (AUC), respectively during oral glucose tolerance test. IRI is the abreviation for insulin resistance index. AUC was calculated geometrically using trapezoidal rule.

2.5 Statistical analysis

Statistical analyses were carried out on Statistical Package for Social Sciences (SPSS for Windows, release 11.0, 2002, SPSS, Chicago, IL, USA). In each study, the data was checked for normality by examining the histograms for any skewness, and by using Q-Q plot. The distribution was considered as symmetrical and normal when the value was very close to zero. Diversion from this condition was defined as non-symmetrical or not normal distribution. When data was found to be normally distributed, the results were expressed as means and standard deviation and parametric statistical test were carried out. In situations where the data was found to be not normally distributed, the results were expressed as median and range, and non-parametric statistical tests were performed. Details of the statistical test used in each study are discussed the relevant chapter. P values of less than 0.05 were considered to be statistically significant. Power calculations were carried out for studies one and three, and the details are given in the chapters.

Chapter 3

Effect of increasing carbohydrate intake (as part of advice to follow the UK dietary guidelines) on metabolic risk factors for CHD in healthy postmenopausal women

3.1 Introduction

Coronary heart disease (CHD) remains the major leading cause of premature death in the world and the global burden of CHD is increasing in association with increasing prevalence of type 2 diabetes mellitus, obesity and metabolic syndrome (FAO/WHO 2003). In fact, CHD mortality data collected by WHO show that the mortality rate in the UK, particularly in Scotland, is one of the highest in the world (The Scottish Office 2000; FAO/WHO 2003). In the UK, CHD resulted in one in four male deaths and one in six female deaths, and caused around 125,000 deaths in 2000. CHD costs the National Health Service around £1.6 billion each year, only 1% of which is spent on primary prevention. The overall cost of CHD to the UK economy is around £10 billion each year (British Heart Foundation, 2002). Although in the UK there are high risk factors for CHD, there are not many published data on the effectiveness of CHD interventions in preventing or reducing these risk factors in the UK, particularly in women.

A number of risk factors for CHD have been identified as reversible and irreversible risk factors (Bittner, 2002). Factors such as age, gender, ethnicity (i.e. African-Americans) and genetic background are non-modifiable risk factors but diet, lack of regular physical activity, obesity, tobacco use, high level of alcohol consumption, dyslipidemia, high blood pressure and diabetes mellitus are among the more than 300 suggested risk factors for CHD and related diseases that could be modified (FAO/WHO, 2003).

Women at any given age are generally at lower risk than men for CHD until their menopause. Women after menopause lose the supportive effects of sex hormones

(Rosenberg *et al.* 1981). Steroids have important biological effects on vascular function and beneficial effects of oestrogens are caused by modulation of lipid metabolism (Suzuki *et al.* 2003). There are three main types of oestrogens in human metabolism:

(1) Oestrone (E1) is an oestrogen formed from oestradiol and is the predominant kind of steroid after menopause.

(2) Estradiol (E2) is the primary oestrogen produced by the ovaries. E2 is a weak oestrogen and the most abundant form found in the body before menopause.

(3) Estriol (E3) is produced in large amounts during pregnancy and is a breakdown product of estradiol. E3 is also a weak oestrogen and may have anti-cancer effects (Mueck *et al.* 2002). Before menopause, estradiol is the predominant oestrogen but after menopause estradiol levels drop more than oestrone. Oestrone would be the predominant oestrogen in postmenopausal metabolism (Seed and Knopp, 2004).

When confounding variables for lipid profile such as body mass index (BMI), smoking and age are matched, the natural menopause is associated with aggravated postprandial lipemia. The progression of higher postprandial lipemia may explain the link between TAG level and CHD mortality risk in postmenopausal women (van Beek *et al.* 1996; Durrington 1998). TAG levels are very important risk factors for CHD (Williams, 2004).

Results from a meta-analysis of 17 population-based studies showed a 76% increase in CHD risk in women and a 31% increase in men associated with a 1 mmol/L increase in TAG levels (Austin *et al.* 1999). The menstrual cycle phase has been reported to have a significant effect on plasma TAG levels but not on plasma cholesterol (Woods *et al.* 1987). When oestrogen levels are high, TAG concentrations are low. After menopause when oestrogen levels decline, LDL cholesterol and TAG increase while HDL cholesterol decreases (Rich-Edwards *et al.* 1995; Tremollieres *et al.* 1996; Jeppesen *et al.* 1997). Oestrogen also influences chylomicron metabolism in postmenopausal women. Deficiency of endogenous oestrogens may lead to a decreased chylomicron clearance capacity (Westerveld *et al.* 1995). This will also increase risk of CHD.

It has been well established that diet plays a prominent role in the aetiology and development of CHD (Hu *et al.* 1997; Williams *et al.* 1999; Hu *et al.* 1999; Joshipura *et al.* 2001; Hu *et al.* 2001; Fung *et al.* 2001; Poulter, 2003). A 'prudent' dietary pattern characterized by higher intakes of fruits, vegetables, legumes, fish, poultry, and whole grains was associated with a lower risk for CHD (RR=0.76, 95%CI: 0.60-0.98) while the 'Western dietary pattern' characterized by higher intakes of red and processed meats, sweets and desserts, french fries, and refined grains significantly increased the risk of CHD (Fung *et al.* 2001). Increased whole-grain intake was associated with decreased risk of CHD after adjustment for age and smoking (Liu *et al.* 1999). The protective effects against CHD have been shown by increasing consumption of fruits and vegetables, particularly green leafy vegetables and vitamin C-rich fruits and vegetables (RR=0.80, 95% CI: 0.69 to 0.93; Joshipura *et al.* 2001). All these results have led

to dietary recommendations for the general public including postmenopausal women.

The current dietary guidelines are to eat a high carbohydrate diet which is low in fat and to consume at least five portions of fruit and vegetables per day. This is supposed to reduce risk of CHD (Ullmann *et al.* 1991; Rimm *et al.* 1996; Dreon *et al.* 1999; Hu *et al.* 1999; Liu *et al.* 1999; Fung *et al.* 2001; Hu *et al.* 2001; Joshipura *et al.* 2001; Cernea *et al.* 2003). However, several studies have shown that a high carbohydrate diet rather than decrease plasma lipids and increase HDL cholesterol actually had the opposite effect in postmenopausal women. For instance, controlled feeding of a high carbohydrate diet in 14 healthy postmenopausal women for 4 months resulted in hypertriglyceridemia but was not associated with a reduction in LDL particle size (Kasim-Karakas *et al.* 1997). The effect of following the American Heart Association Step 1 diet (i.e. low fat, low saturated fat, low cholesterol and high carbohydrate diet) for 10 weeks among 55 overweight and obese postmenopausal women showed reductions in body weight, total cholesterol, LDL and HDL cholesterol concentrations (Bunyard *et al.* 2002). The effect of variation in carbohydrate and fat intake using two experimental isoenergetic diets (i.e. 60% and 25% *vs* 40% and 45% energy from carbohydrate and fat, respectively) was investigated in postmenopausal women (each diet for three weeks). Results showed that fasting TAG and VLDL cholesterol concentrations were higher after the high-carbohydrate diet while HDL cholesterol level was lower compared with the high-fat diet (Jeppesen *et al.* 1997). These studies are mostly very short term and the effects may not be sustained over a longer time.

When a high carbohydrate diet is followed by an individual under free living conditions, they can chose from simple and complex carbohydrates. The proportion of sugars eaten may be important in determining the plasma lipid profile as sugars have been reported to have more adverse effects on TAG than other forms of carbohydrate such as starch (Parks and Hellerstein, 2000) and high fructose intake has also been related to increased TAG (Gross *et al* 2004). This may also be related to the GI of the diet chosen and several epidemiological studies have suggested that high GI diets increase the risk factors for CHD (Liu *et al.* 2000) and type 2 diabetes (Salmeron *et al.* 1997a; Salmeron *et al.* 1997b) and in the control of risk factors for these diseases (Frost *et al.* 1999; Ford & Liu, 2000; Liu *et al.* 2001; Brand-Miller *et al.* 2003). However, not all studies have shown that GI is important in risk of chronic disease (van Dam *et al.* 2000).

Thus the way that an individual interprets the guidelines may be as important in determining their lipid profile as a choice to change their diet. It is therefore very important to consider the effects of the guidelines under free living conditions to take this into account.

In summary, it is not clear whether complying with current dietary guidelines makes postmenopausal women more susceptible to CHD. There is no published evidence on the effects of following UK dietary guidelines on metabolic risk factors for CHD in free-living healthy postmenopausal women. In the study described in this chapter, therefore, the effect of dietary advice based on the UK recommendations on plasma lipids was investigated in free-living postmenopausal women over 4 weeks

3.2 Objectives

To investigate the effect of advice to increase carbohydrate intake, as part of advice to follow the UK dietary guidelines, on metabolic risk factors for CHD in healthy free-living postmenopausal women

3.3 Materials and methods

In this study, I organised the day-to-day running of the study, carried out the analysis of total and HDL cholesterol, triaclyglycerol, glucose and ferric reducing ability of plasma (FRAP) assay, the dietary (Diet 5) analysis, calculations of GI and GL and statistical analysis.

3.3.1 Subjects

Twelve subjects were recruited to take part. However, two subjects dropped out after the first week of the intervention and did not complete the whole study. Therefore, the subject numbers are 12 for baseline and week 1 measurements, and 10 for the week 4 measurements.

The volunteers, apparently healthy postmenopausal women, were recruited by various methods including: an advertisement in the university newsletter and in a local newspaper, posters, and via friends and family of Department of Human Nutrition employees. The study received ethical approval from Glasgow University Ethics Committee and written informed consent was obtained from each volunteer before being enrolled into the study.

To take part in the study, subjects were required to fulfil a number of criteria including: to be healthy, to be postmenopausal, to have had their last menses at least three years before starting the study, not to have had a hysterectomy or ovary surgery, not receiving hormone replacement therapy (HRT), not to be obese (i.e. BMI less than 30 $kg.m^{-2}$, not taking any medication known to affect carbohydrate or lipid metabolism, not trying to lose weight, taking any antibiotics or dietary supplements.

As subjects were required to be healthy they completed a health history questionnaire.

3.3.2 Experimental design

This study was a four-week dietary advice intervention of free-living subjects with measurements taken at baseline, after one week and four weeks of the dietary intervention. Subjects were involved in the study for approximately five weeks in all. Subjects attended the Department of Human Nutrition at Yorkhill Hospital for four visits including: at the screening, baseline, end of the first week, and at the end of week four of the dietary intervention.

At the screening visit, subjects were given the opportunity to ask questions about the study before signing the consent form. The information sheet was posted to potential subjects in advance to give them time to read it and to discuss with family and friends, if necessary. At this visit, subjects provided their contact details, the inclusion criteria for the study were checked and subjects completed

the health history questionnaire. In addition, anthropometric measurements were carried out and a fasted blood sample was taken. At this visit, subjects were asked to carry out a 7-day weighed intake of their habitual dietary intake. It was stressed that subjects should try as much as possible not to change from their usual dietary habits and that this measurement should reflect their habitual diet as much as possible. Subjects were provided with oral and written instructions, a digital food scales and an A3-sized diary, which they were asked to post back to the Department for analysis before the next visit. Subjects were provided with a simple breakfast before leaving the study room.

Subjects were required to attend the baseline visit usually within a couple of days of receiving the returned completed 7-day weighed intake dairy to allow for time to analysis the diary and preparation of the individualised dietary advice. Subjects were required to attend this visit after at least a 12 hour fast and anthropometric measurements were taken and a fasted blood sample obtained before the subject was provided with a light breakfast before leaving. At this visit, subjects were shown the results of the habitual dietary intake compared to the current dietary reference values (Department of Health, 1991) and advice on the basis on this on how to make practical changes to their diet in order to better comply with the recommendations. Subjects were asked to follow this advice for four weeks in a free-living situation and were not provided with food or funds to purchase particular foods. During the dietary intervention period subjects were required to keep a 3-day dietary diary (one weekend and two weeks days) of all food and beverages consumed. This was used to assess compliance to the dietary advice given.

Subjects were required to return after one week and after four weeks of following the dietary advice to give a fasted blood sample and to have anthropometric measurements taken. During the visit after the first week of the dietary intervention, subjects were required to bring their 3-day diet diary for inspection and were encouraged to maintain the dietary changes for a further three weeks. Subjects were given three more 3-day diet diaries to complete during each subsequent week of the dietary intervention with stamped addressed envelopes to post back to AH for analysis. Subjects were contacted a number of times during the subsequent three weeks via email and post and encouraged to keep following the dietary advice given at the baseline visit.

3.3.3 Anthropometric measurements

Anthropometric measurements were made in the fasted state, in light clothing and without shoes using. Body weight was measured at each visit while height, waist circumference, hip circumference, mid-upper arm circumference and body fat were measured at the screening and baseline visits only. Weight was measured with a digital scales (SECA scales, Germany), height (m) using a stadiometer (Holtain Ltd, Crymych, Dyfed) with subjects in a relaxed position and arms hanging freely. Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m^2). Waist, hip and mid-upper arm circumferences were measured by standard recommended procedures (described in the methods chapter). Body fat was estimated by skinfold thickness using calipers (Holtain Ltd. Crymych, UK). Skinfold thickness measurements were taken at four different sites; biceps; triceps; subscapular; suprailliac. Three measurements were taken at each site and averaged, and the sum of the four values was used to calculate the % body fat of each subject using the equations of Durnin & Wormersley, (1974).

3.3.4 Dietary intervention

Dietary targets for 2005 in Scotland (The Scottish Office, 1993) have been defined:

1) To increase consumption of starchy foods such as breads, cereals, potatoes (>40% of energy),
2) To increase non-starch polysaccharides e.g. fruits and vegetables (>16g),
3) Reduction in total and saturated fat intake (less than 35% and11% of energy, respectively) by consumption of low fat products and decreasing intake of high-fat foods such as chips.

Table 3.1 Department of Health (1991), Scottish Diet Report and Dietary Targets for 2005

Macronutrient	Current average intake	Proposed average
% Energy from:		
Carbohydrates		
Starch	25.3	>40
NSP (g)	10.5	>16
Sugar	16.3	<10
Total fat	40.7	<35
Saturated fat	16.6	<11

Subjects were advised to alter their habitual diet to include 55%, 30% and 15% of from carbohydrate, fat and protein, respectively. The subjects were advised:

1) To consume more complex carbohydrates especially more starchy foods such as pasta, boiled rice, boiled and baked potatoes and breakfast cereals,
2) To replace the intake of saturated fat with carbohydrate,
3) NSP more than 16g/day,

4) - To avoid fried and high-fat foods and high intake of saturated fatty acids (Details in Table 3.2)

Each subject was advised on their diet in an individual manner based on their habitual diet records,

3.3.5 Dietary assessment

During the course of this study, subjects were asked to carry out a 7-day weighed intake to assess their habitual diet prior to starting the dietary intervention and were also asked to carry out four 3-day weighed intakes during each week of the four weeks of the dietary intervention. Subjects were asked keep a record of all foods and drinks consumed and asked to weigh each food item immediately prior to consumption. Subjects were provided with a portable electronic food scales (Slater Household Ltd, Tonbridge, UK), a food diary (with the appropriate number of pages depending on whether they were required to record food intake for seven or three days). Subjects were provided with both verbal and written instructions on how to record their food and beverage intake, and on correct use of the scales. At the baseline visit, subjects were shown how to use the digital scales including use of the 'tare' facility to allow weighing of foods cumulatively on to a plate. The completed diet records were inspected on their return to ensure that the subjects' diaries were complete, and that sufficient detail had been recorded.

Table 3. 2. Dietary goals and practical advice given to achieve these goals

Goals	Advice
Increase intake of carbohydrate (55% of total energy intake)	Increase intake of starchy foods (e.g. breads, breakfast cereals, potatoes, pasta, rice), fruits, vegetables, legumes

Increase intake of more complex carbohydrates such as starchy foods (>45% of total energy)	Increase consumption of breads, particularly wholemeal and brown breads, cereals and potatoes, such as baked/mashed/boiled potatoes, pasta, filling lettuce, tomato, cucumber or cooked vegetables
Decrease intake of simple sugar (<10% total energy)	Decrease intake of sugar, sweets or food products with added sugar
Increase intake of non-starch polysaccharides (>16g/day)	Eat fruit and vegetables daily (5 portions of fruit and vegetables including fresh, frozen, canned, or dried fruits, vegetables and pulses)
	Increase fruit and vegetable intakes to at least 400g per day
	Choose less-refined breads and cereals
Reduce intake of total fat and saturated fatty acids (less than 30% and 10% of total energy, respectively)	Choose low-fat milk, low-fat cheese, low-fat meat and meat products (e.g. lean cooked ham, chicken, beef or turkey)
	Choose steamed, boiled, grilled or micro-waved meat and fish
	Decrease intake of fried foods (e.g. French fries) and high-fat meat products (e.g. sausages and meat pies)
	Choose oily fish such as salmon, tuna

Dietary analysis was carried out using a computerised version of Dict 5™ (Robert Gordon University, Aberdeen) of the food composition tables (Holland *et al.* 1991). Diet diaries were analysed for daily energy and macronutrient intakes in absolute amounts as well as the percentage of energy derived from the main macronutrients.

Subjects' habitual diet records were not included in the statistical analysis if their reported energy intakes were less than their estimated basal metabolic rate (BMR)

multiplied by 1.1 (under-reporters) or greater than 2.0 (over-reporters). BMR was estimated using the Scofield equations (Schofield *et al.* 1985). These cut-offs are used as it is highly unlikely that habitual energy intake would be < 1.1 × BMR or > 2.0 × BMR (Goldberg *et al.* 1991). One subjects' diet records were excluded as she was found to have under-reported.

3.3.6 Calculation of GI and GL

GI and GL were estimated from subjects' 7-day and 3-day weighed intake diaries. The GL of each carbohydrate-containing food recorded in the diet diary was estimated by multiplying the carbohydrate content of the serving of food (obtained from Diet 5) by the GI of that food item (obtained from the International Table of GI and GL; (Foster-Powell *et al.* 2002) divided by 100 (Brand-Miller *et al.* 2003). The GL values, calculated in this way, for each carbohydrate-containing food consumed was summed to give the GL for seven or three days, and this value was then divided by seven or three to give the daily GL. The daily GI value was then calculated by dividing the daily GL by the total daily carbohydrate intake (obtained from Diet 5), and by multiplying by 100 (Brand-Miller *et al.* 2003).

3.3.7 Blood Sampling

A fasted blood sample was obtained from subjects at each of the four visits. Subjects were advised to come to the laboratory early in the morning (before breakfast) and after fasting for at least 12 hours beforehand. Subjects were asked to not to consume any alcohol or to participate in any vigorous physical activity for at least 24 hours before this visit. A venous blood sample was taken from the

ante-cubital fossa vein in the non-dominant arm into vacutainers containing EDTA and sodium fluoride. The blood samples were immediately placed on wet ice and centrifuged at 3,000 rpm for 10 minutes at 4° using clinical centrifuge (MSE Leicester, U.K.). Plasma was divided into pre-labelled aliquots and stored at -80°C.

Blood samples taking in EDTA vacutainers were used for lipid, insulin, C-reactive protein and non-esterified fatty acid concentration analysis while blood samples taking into sodium fluoride were used for the analysis of glucose concentrations.

3.3.8 Laboratory analysis

The full details of the methods used for these laboratory analyses are described in Chapter 2, and therefore only a brief indication of the method is given here. Total cholesterol concentration was determined using a coloromctric enzymatic assay (SIGMA Chemical Co., St. Louis, U.S.A) with the coefficient of variation of 1.63%. HDL cholesterol concentration was assessed using the same kit after the precipitation of apolipoprotein B-containing lipoproteins with magnesium-chloride and sodium-tangestate mixture at 500g for 10 min. by micro-centrifuge (MSE micro-centrifuge, UK). The coefficient of variation for the measurement of HDL cholesterol was 2.78%. Fasting triaclyglycerol concentrations were determined coloromtrically (SIGMA Chemical Co., St.Loius, U.S.A) with a coefficient of variation 2.2 %. LDL cholesterol concentrations were estimated using the Friedewald equation (Friedewald *et al.* 1972) as follows:

LDL cholesterol ($mg.dl^{-1}$) = Total cholesterol – (HDL cholesterol ($mg.dl^{-1}$) + Fasting triaclyglycerol ($mg.dl^{-1}$)/5)

Insulin concentrations were determined at the Biochemistry Department at the Glasgow Royal Infirmary using an automated analyser technique (Abbot IMX Analyser and dry slice technology. The homeostatic model assessment ($HOMA_{IR}$) technique (fasting insulin x fasting glucose/22.5) was used as a validated surrogate measure of insulin resistance (Mattews *et al.* 1985). Analysis of C-reactive protein (CRP) was carried out in EDTA plasma samples using an in house ELISA (Highton & Hessian, 1984) at the Department of Pathological Biochemistry at the Glasgow Royal Infirmary.

3.3.9 Ferric reducing ability of plasma assay (FRAP)

The antioxidant power of plasma was determined using the ferric reducing ability of plasma (FRAP) assay, as described by Benzie & Strain (1996). This method assesses the 'antioxidant power' by measuring the absorbance (i.e. optical density) of the blue colour developed when a ferric-tripyridyl-triazine (Fe^{3+}-TPTZ) complex is reduced to the ferrous form (Fe^{2+}). The greatest 'antioxidant power' is associated with the deepest shades of blue. Briefly, the method involves adding 3 ml of FRAP reagent (ferrous chloride, TPTZ and acetate buffer at pH 3.6, made freshly each day) and 400 µl water into individual test tubes (in triplicate). Then at 30-second intervals, 100 µl EDTA plasma was added to each test tube and vortex mixed for 30 seconds and incubated for to exactly 4 minutes before the absorbance was read. The absorbance of the samples was then determined at 593 nm using spectrophotometer (Thermo Labsystems Mulitiscan,

Vaanta, Finland) The FRAP (mmol Fe^{2+}.L) concentrations of the samples were calculated against a standard curve ranging from 0.1-1.0 mmol/L of ferrous sulphate and results are expressed as mmol Fe^{2+}/L of ferrous iron formed.

The obtained coefficient of variation for the assay was 2.7 %.

3.3.10 Statistical methods

The computerised statistical package SPSS (version 11.0) was used to perform the statistical analysis. All data was checked for normality by visual inspection of histograms and comparison between mean and median in SPSS. As data was not normally distributed, Data are given as median and range. Wilcoxon Sign ranked test was used to compare the difference in dietary data between habitual and after one week and also habitual and mean of four weeks of dietary intervention trial. Similarly, metabolic risk factors were compared at two time sections (i.e. baseline and after one week as well as baseline and at the end of four weeks dietary intervention trial). Spearman's correlation test was used to explore the relationships between dietary and metabolic parameters. Statistical significance was accepted at the $P<0.05$ level.

3.4 Results

3.4.1 Subject characteristics

Postmenopausal women (n=12) in this study were aged from 46 to 66 years old and their last period was between 3 to 16 years before joining this study. The mean BMI was 24.7 $kg.m^{-2}$, however the range was 21.6 to 29.3 for study group. Waist circumferences and waist to hip ratio were determined for four subjects only in this study (Table 3.3).

Table 3.3. Subject characteristics (n=12)

Characteristics	Mean	SD	Range
Age (years)	56.2	6.5	46-66
BMI ($kg.m^{-2}$)	24.7	2.8	21.6-29.3
Waist circumference (cm)*	76.0	7.8	66.0-85.0
Waist to hip ratio*	0.73	0.06	0.66-0.78
Last period (years)	7.1	5.0	3-16

* n=4

Table 3.4. Daily energy and macronutrient intakes of habitual diet and of the first week and a mean of four weeks of the dietary intervention in postmenopausal women by weighed diet record. Values are medians and inter-quartile ranges (Q1, Q3).

	Habitual diet (n=11)		Week one (n=11)		*P**	Median of four weeks (n=9)		*P***
	Median	Q1, Q3	Median	Q1, Q3		Median	Q1, Q3	
Energy (kj)	8494	(7719,10205)	7142	(6343,8000)	**0.021**	6576	(5843,7716)	**0.011**
Energy (kcal)	2030	(1845,2439)	1707	(1516,1912)	**0.021**	1572	(1397,1844)	**0.011**
Nutrient (g)								
Carbohydrate	215.5	(193.5,289.3)	236.3	(194.6,262.4)	0.657	213.0	(185.0,256.0)	0.260
Sugar	92.6	(77.8,152.7)	99.8	(73.5,103.2)	0.477	87.0	(75.7,113.8)	0.110
Non-milk extrinsic sugar	69.4	(45.2, 98.9)	55.5	(37.0,76.4)	0.131	53.2	(41.5,60.0)	**0.015**
Starch	105.9	(87.3,137.1)	137.4	(87.6,147.6)	0.131	114.4	(104.3,139.4)	0.594
Sugar: starch ratio	0.82	(0.63, 1.2)	0.74	(0.69,0.84)	0.11	0.71	(0.65,0.87)	0.374
Non-starch polysaccharides	12.8	(10.7,15.4)	17.2	(14.9,21.3)	**0.050**	16.4	(13.9,19.7)	0.086
Total fat	80.8	(71.1,98.2)	50.5	(39.9,64.0)	**0.013**	43.6	(41.3,53.0)	**0.011**
Saturated fat	29.6	(20.9,44.2)	15.6	(10.2,19.0)	**0.026**	13.6	(12.2,15.3)	**0.011**
Monounsaturated fat	26.6	(22.1,33.4)	15.7	(13.0,17.5)	**0.026**	14.1	(13.6,16.7)	**0.011**
Polyunsaturated fat	11.6	(9.5,17.7)	8.7	(8.0,10.8)	0.075	9.3	(7.4,10.0)	**0.021**
Protein	84.9	(71.8,112.1)	74.7	(47.4,95.0)	0.158	73.4	(62.8,80.6)	**0.003**
Alcohol	14.3	(7.4,33.9)	14.6	(9.0,22.7)	0.824	9.9	(6.3,17.0)	0.260

*** *P* for comparison between habitual diet and week one of the dietary intervention trial (Wilcoxon Signed Ranks test)**

**** *P* for comparison between habitual diet and mean of four weeks of the dietary intervention trial (Wilcoxon Signed Ranks test)**

3.4.2 Daily energy and macronutrient intakes (absolute amount)

Table 3.4 illustrates the daily energy and macronutrient intakes in absolute amounts in the habitual diet, after one and four weeks of the dietary intervention of the postmenopausal women. There was a significant reduction in daily energy intake (kcal and kj) during the first week (P=0.021) and after a mean of four weeks (P=0.011) of the dietary intervention.

There was a reduction in non-milk extrinsic sugar intake following a mean of four weeks of the dietary intervention (P=0.015).

On the other hand, there was an increase in non-starch polysaccharide intake, which was statistically significant after one week (P=0.05), but not quite statistically significant after a mean of four weeks of dietary intervention (P=0.086).

There was significant reduction in total fat intakes after one week (P=0.013) and after a mean of four weeks (P=0.011), saturated and monounsaturated fat intakes after one (P=0.026) and four weeks (P=0.011). Polyunsaturated fat intake was not significantly reduced after one week (P=0.075) but was significantly reduced after a mean of four weeks of the dietary intervention (P=0.021) (Table 3.4).

There was a significant reduction in protein intake following a mean of four weeks of the dietary intervention (P=0.003).

Table 3.5. The percentage of energy intake from the macronutrients of habitual diet and during the first week, and after a mean of four weeks of the dietary intervention in postmenopausal women by weighed diet record. Values are medians and inter-quartile ranges (Q1, Q3).

	Habitual diet (n=11)		Week one (n=11)		*P**	Mean of four weeks (n=9)		*P***
	Median	Q1, Q3	Median	Q1, Q3		Median	Q1, Q3	
% Energy from:								
Carbohydrate	40.1	(35.1,48.9)	48.6	(43.4,53.9)	**0.026**	51.0	(47.2,55.4)	0.051
Sugar	18.1	(14.8,22.1)	20.2	(17.0,24.4)	0.328	21.6	(19.6,25.4)	0.208
Non-milk extrinsic sugar	11.9	(11.3,15.7)	11.7	(6.8, 14.4)	0.168	12.6	(9.9,14.0)	0.374
Starch	20.8	(16.3,34.2)	30.8	(18.5, 37.4)	**0.002**	29.2	(25.7,35.8)	**0.013**
Total fat	32.6	(29.6,41.1)	27.5	(20.8, 30.2)	**0.008**	25.5	(24.4, 26.7)	**0.008**
Saturated fat	13.0	(9.6,16.3)	6.0	(5.7, 8.4)	**0.004**	7.8	(6.7, 8.6)	**0.008**
Monounsaturated fat	11.3	(9.9,14.2)	6.7	(6.0, 8.7)	**0.003**	8.0	(7.5, 8.6)	**0.008**
Polyunsaturated fat	6.1	(4.9,7.8)	4.1	(3.5, 5.9)	0.075	4.6	(3.9, 5.6)	0.173
Protein	15.6	(13.6,23.7)	16.9	(13.1,25.8)	0.065	17.7	(14.4,22.1)	0.114
Alcohol	3.6	(2.5,9.8)	5.4	(2.5,10.2)	0.859	5.3	(2.1,7.1)	0.767

*** *P* for comparison between habitual diet and after week one of the dietary intervention trial (Wilcoxon Signed Ranks test)**
**** *P* for comparison between habitual diet and after mean of four weeks of the dietary intervention trial (Wilcoxon Signed Ranks test)**

3.4.3 Percentage of energy intake from macronutrients

The subjects significantly increased their percentage of energy from carbohydrates and starch (P=0.026 and P=0.002, respectively) during week one of the dietary intervention, and there was a significant increase in the percentage of energy from starch after a mean of four weeks of the dietary intervention (P=0.013) (Table 3.5).

There was a significant decrease in percentage of energy from total fat (P=0.008), saturated fat (P=0.004 and P =0.008) and monounsaturated fat (P =0.003 and P =0.008) during week one and after a mean of four weeks of the dietary intervention, respectively (Table 3.5).

3.4.4 Dietary glycaemic load (GL) and glycaemic index (GI)

GI was significantly increased after week one (P=0.026) and after a mean of four weeks of the intervention (P=0.011) (Table 3.6).

When GL and GI were expressed per 1000 kcal, GL (P=0.011 and P=0.003) and GI (P=0.008 and P=0.011) were significantly increased after one week and after a mean of four weeks of the dietary intervention (Table 3.6).

Table 3.6 Dietary GI and GL of the habitual diet and during week one, and after a mean of four weeks of the dietary intervention in postmenopausal women by weighed diet record. Values are medians and inter-quartile ranges (Q1, Q3).

	Habitual diet (n=11)		Week one (n=11)		*P**	Mean of four weeks (n=9)		*P***
	Median	Q1, Q3	Median	Q1, Q3		Median	Q1, Q3	
Glycaemic load (g)	100	(98,129)	119	(105,152)	0.062	117	(103,152)	0.374
Glycaemic index (%)	48	(45,52)	58	(49,60)	**0.026**	58	(54,59)	**0.011**
Per 1000 kcal:								
Glycaemic load (g)	53	(45,64)	82	(66,86)	**0.011**	75	(64,79)	**0.003**
Glycaemic index (%)	23	(21,25)	32	(30,39)	**0.008**	35	(31,39)	**0.011**

*** *P* for comparison between habitual diet and during week one of the dietary intervention trial (Wilcoxon Signed Ranks test)**

**** *P* for comparison between habitual diet and after mean of four weeks of the dietary intervention trial (Wilcoxon Signed Ranks test)**

Table 3.7. BMI and biochemical parameters at baseline and after one and four weeks of dietary intervention in postmenopausal women. Values are medians and inter-quartile ranges (Q1, Q3).

	Baseline (n=12)		After one week (n=12)		*P**	After four weeks (n=10)		*P***
	Median	Q1, Q3	Median	Q1, Q3		Median	Q1, Q3	
BMI ($kg.m^{-2}$)	24.6	(22.7,28.2)	24.6	(22.5,28.3)	0.155	23.1	(22.4,27.4)	**0.014**
Triacylglycerol ($mmol.l^{-1}$)	0.95	(0.84,1.06)	1.01	(0.94,1.14)	**0.014**	1.02	(0.82,1.35)	0.083
Total cholesterol ($mmol.l^{-1}$)	5.22	(4.75,5.78)	5.10	(4.67,5.74)	**0.117**	5.00	(4.74,5.85)	0.333
HDL cholesterol ($mmol.l^{-1}$)	1.72	(1.52,1.95)	1.67	(1.48,1.80)	**0.014**	1.46	(1.38,1.64)	**0.021**
LDL cholesterol ($mmol.l^{-1}$)	3.14	(2.60,4.00)	2.96	(2.45,4.12)	0.147	2.88	(2.30,4.12)	0.308
TC:HDL cholesterol ratio	3.04	(2.52,3.98)	3.00	(2.56,4.03)	0.754	3.29	(2.66,4.16)	0.114
LDL particle size (nm)	25.85	(25.70,25.93)	25.81	(25.68,25.91)	**0.004**	25.71	(25.46,25.83)	**0.028**
Fasting glucose ($mmol.l^{-1}$)	5.08	(4.79,5.38)	5.17	(4.68,5.34)	0.784	5.16	(4.72,5.55)	0.386
Fasting insulin ($\mu U.ml^{-1}$)	4.50	(2.82,6.98)	4.95	(4.35,6.12)	0.789	5.60	(3.75,6.98)	0.333
$HOMA_{IR}$	1.09	(0.59,1.56)	1.11	(0.90,1.12)	0.937	1.25	(0.83,1.72)	0.333
C-reactive protein ($mg.l^{-1}$)	0.42	(0.33,1.77)	0.60	(0.40,1.05)	0.625	0.46	(0.26,1.43)	0.953
FRAP ($mmol.l^{-1}$)	0.55	(0.50,0.59)	0.56	(0.55,0.63)	**0.002**	0.57	(0.53,0.60)	**0.007**

*** *P* for comparison between baseline and after one week dietary intervention (Wilcoxon Signed Ranks test)**

**** *P* for comparison between baseline and after four weeks dietary intervention (Wilcoxon Signed Ranks test)**

Table 3.8 Normal ranges and recommended cut-offs for high levels of blood lipids

Lipid	Level	Concentration (mmol/L)
Total cholesterol	**Desirable**	<5.2
	Borderline high	5.2-6.2
	High	>6.2
LDL cholesterol	**Optimal**	<2.6
	Near or above optimal	2.6-3.3
	Borderline high	3.4-4.1
	High	4.2-4.9
	Very high	>4.9
HDL cholesterol	**Low**	<1.0
	High	≥1.6
Triacylglycerol	**Normal**	<1.7
	Borderline high	1.7-2.3
	High	2.4-5.6
	Very high	>5.6

Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (2001)
Cholesterol concentrations were converted from mg/dL to mmol/L by dividing by 38.7
Triacylglycerol concentrations were converted from mg/dL to mmol/L by dividing by 88.6

3.4.5 BMI and biochemical factors

There was no significant change in BMI after one week of dietary intervention (P=0.155) but there was a significant decrease after four weeks of dietary intervention (P=0.014; Table 3.7).

There was a significant increase in fasting triacylglycerol concentrations after one week of dietary intervention (P=0.014) but this difference did not reach statistically significance after four weeks of dietary intervention (P=0.083).

HDL cholesterol concentration showed a significant decrease after one week (P=0.014) and also after four weeks of the dietary intervention (P=0.021). There was no statistically significant change in total and LDL cholesterol concentrations after one and four weeks of dietary intervention. LDL particle size was significantly decreased after one and four weeks of dietary intervention (P=0.004 and P=0.028, respectively).

There was no statistically significant change in fasting glucose and insulin concentrations after one and four weeks of dietary intervention. Although HOMA score showed an increase after four weeks of dietary intervention but it was not statistically significant (P=0.333).

Table 3.8 show the normal ranges and recommended cut-offs for high levels of blood lipids.

3.4.6 Correlations between simple and complex carbohydrates and plasma lipids

The relationships between starch intake and sugar intake with plasma lipids was explored to see if the type of carbohydrate was important in determining the lipid response.

There was a significant positive association between change in energy from simple sugars and the change in TC/HDL-C ratio (r=0.66, P=0.029) after 1 week (Figure 3.1). There was also a significant relationship between the percent of changes in sugar to starch ratio intake and the percent of changes in TC/HDL-C after four weeks (r = 0.67, P = 0.050) (Figure 3.2). No statistically significant relationships were found between the percentage change in GI and GL and fasting lipid concentrations. Furthermore, no statistically significant relationships were found between the percentage change in fasting lipid concentrations and percentage change in dietary intakes of fat.

Figure 3.1 Relationship between changes in energy from sugar intake and changes in TC/HDL-C ratio in 11 postmenopausal women after dietary advice

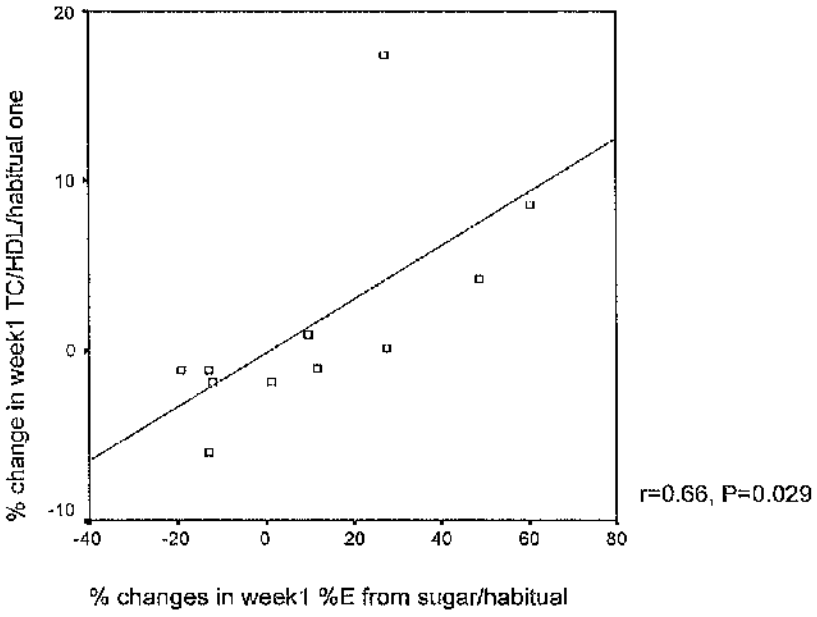
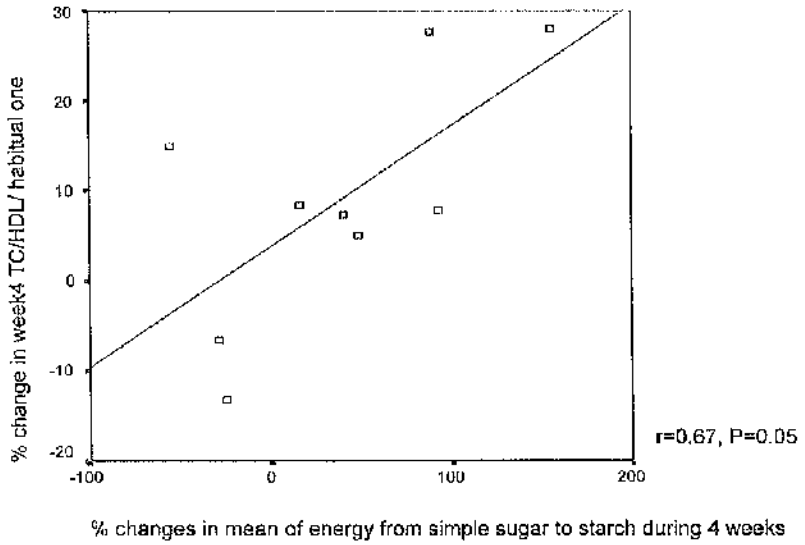


Figure 3.2 Relationship between the percent of changes in sugar to starch ratio intake and the percent of changes in TC/HDL-C ratio in 9 postmenopausal women after four weeks dietary advice



3.4.7 Changes in FRAP and CRP

There was a significant increase in the antioxidant potential of the plasma as measured by FRAP (Tables 3.7) and this was related to the increase in intake of fruit and vegetables (Figure 3.3, Table 3.9). There was no significant effect on C-reactive protein (Table 3.7). However, it is not likely that the study was sufficiently powered to detect a change.

Figure 3.3 The percent change in FRAP capacity and changes in fruit and vegetable intake in 9 postmenopausal women after dietary advice

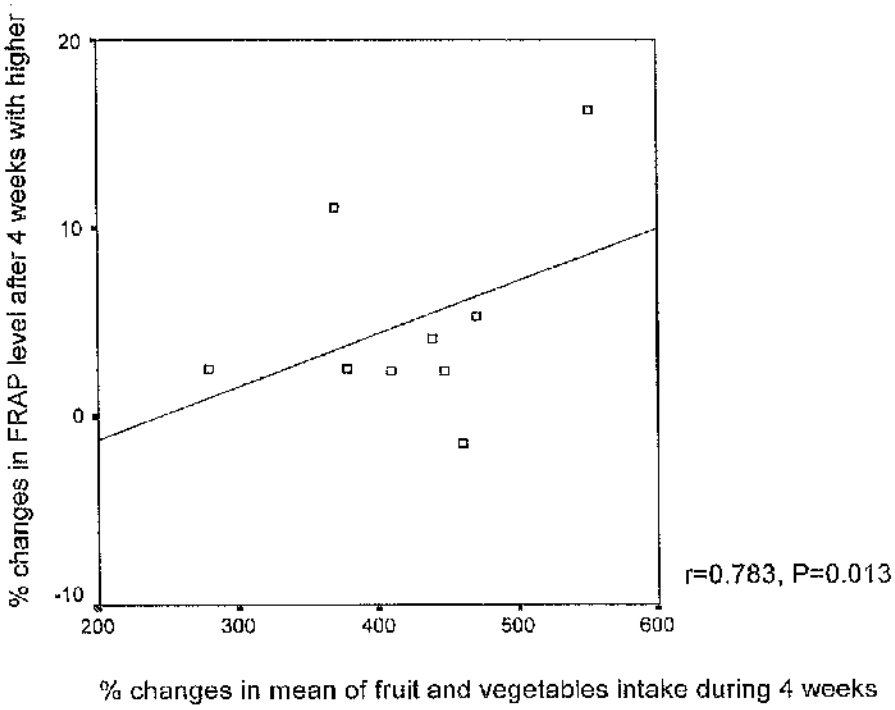


Table 3.9 Effect of changing diet after dietary guideline based advice on fruit and vegetable intake and plasma FRAP (n = 11)

	Habitual (*n* 11)		**After 1 week (*n*=11)**		***P****	**After 4 weeks (*n*= 9)**		***P****
	Median	Range (Q1, Q3)	Median	Range (Q1, Q3)		Median	Range (Q1,Q3)	
Vegetables (g)	50	28, 64	65	35, 98	0.374	64	20, 89	0.860
Fruit (g)	192	133, 325	334	238, 497	**0.016**	374	289, 430	0.139
Fruit & vegetables (g)	220	164, 397	442	300, 535	**0.008**	438	373, 465	0.173
FRAP (mM)	0.55	0.50, 0.59	0.56	0.55, 0.63	**0.002**	0.57	0.53, 0.60	**0.007**

Difference between habitual and week 1; ** Difference between habitual and 4 weeks by Wilcoxon rank test.

3.5 Discussion

In order to reduce the risk of atherosclerosis the current dietary guidelines recommend the replacement of a considerable amount of dietary fat with carbohydrate (Department of Health, 1991). Most studies on the effect of high carbohydrate diets on CHD risk factors have been carried out in short term very controlled laboratory style studies. The aim of this intervention study was to assess the effect of giving dietary advice based on current guidelines in a more normal and relevant situation for free-living postmenopausal women, as a high risk group for CHD. The way they changed their diet was noted in relation to starch and sugar intake, GI and GL and related to the changes in their plasma lipid profiles.

The findings of the present study revealed that the women changed their diet with significant increases in dietary GL and GI and there were significant increases in plasma TAG and FRAP levels and reductions in HDL cholesterol concentration and LDL particle size.

Subjects significantly reduced the absolute amount and percentage of energy intake from total fat, saturated fat, MUFA after one and four weeks of dietary intervention. Consequently, they increase their total carbohydrate and starch intake. Energy from carbohydrates significantly increased after one week but because two subjects dropped out between week one and week four of the dietary intervention, this was not statistically significant after the four-week dietary intervention. The increase in carbohydrate intake was achieved with significant increases in dietary GI after one and four weeks. The dietary GL was non-significantly increased ($P = 0.062$) after one week but was not statistically significantly increased at 4 weeks.

The subjects were advised to increase their carbohydrate intake particularly in respect of starchy foods. Although they significantly decreased the absolute amount of non-milk extrinsic sugar intake after four weeks of dietary intervention, the change was not statistically significant in terms of the proportion of energy. The decrease in body weight over the dietary intervention resulted from a significant reduction in energy intake during the first week and after a mean of four weeks of dietary intervention. However, although they were advised to maintain their normal lifestyle including exercise, it cannot be ruled out that an increase in physical activity was a reason for the weight loss. There was a significant increase in energy from carbohydrates only after one week. The lack of a statistically significant change in energy from carbohydrate at the end of intervention trial may have resulted from the dropping out of two subjects after following one week of the dietary intervention. Energy from starch showed a significant increase after one and four weeks of the dietary intervention. Although the postmenopausal women increased starch intake and decreased the ratio of sugar to starch (0.82 in habitual diet *vs* 0.71 at the end of study), the change in sugar to starch ratio was not statistically significant over the dietary intervention. The mean of sugar intake increased from 19.1% at habitual diet to 21.2%, and 21.6% after one and four weeks of dietary intervention. This reveals that the subjects increased dietary carbohydrates by increasing the intake of both sugar and starch. This is not surprising when dietary advice is given to individuals in a free-living state, which is not under the control of investigator and is more relevant to real life than when complex carbohydrates are given under laboratory style conditions. It reflects the need to be more specific in dietary guidelines and the need to explain more the differences in the types of carbohydrates in foods and their probable effects on health.

The advice to increase carbohydrate intake was also associated with significant increases in dietary GI and GL, which from the literature would be associated with increased risk of CHD (Liu *et al.* 2000) and type 2 diabetes (Salmeron *et al.* 1997a and b) and a worsening of metabolic risk factors (Frost *et al.* 1999; Ford & Liu; 2000; Liu *et al.* 2001) for these diseases.

There was an adverse effect on fasting plasma lipids including an increase in fasting TAG (P = 0.014 and P = 0.083 after one and four weeks, respectively), and a decrease in HDL cholesterol concentrations (P = 0.014 and P = 0.021 after one and four weeks, respectively). A number of correlations were carried out to assess the relationships between changes in the type of carbohydrate eaten and the relative changes in plasma lipid levels (Figures 3.1, 3.2, 3.3). In general there was a protective effect of increasing intakes of complex carbohydrates and starch on plasma lipids and an adverse effect of increases in simple sugar intake. This approach of looking at the relative changes in individuals with their individual lipid profile was more powerful in detecting these changes than looking at group changes alone.

Although the median change for TAG between baseline (0.95 mmol/L) and week 1 (1.01 mmol/L) is statistically significant ($P = 0.014$), looking at the normal ranges and recommended cut-offs for high plasma lipids in Table 3.8, one can see that the changes are still within the normal range (<1.7 mmol/L). However, the trend is for TAG to increase which is not in a benefical direction, and even though the changes might not be clinically significant for the individuals in this study, on a population wide basis, it is very likely to be associated with increased risk of CHD. After all the

results from a meta-analysis of 17 population-based studies showed a 76% increase in CHD risk in women and a 31% increase in men associated with a 1 mmol/L increase in TAG levels (Austin *et al.* 1999). On the other hand, median HDL-C concentrations decreased statistically significantly (P = 0.021) from 1.72 mmol/L to 1.46 mmol/l which meant that the median value at baseline would be considered high (≥ 1.6 mmol/L) which is associated with lower risk of CHD, where as the value after four weeks of the intervention would no longer be categorised as high. Regarding the individual changes in HDL-C, for four of twelve subjects, their HDL-C values decreased from above 1.6 mmol/L to less than this value, which would be considered biologically significant for these individuals. It has also been reported that for every decrease of about 0.026 mmol/L in HDL-C concentration, the risk of CHD increases by 3.2% in women and 2.3% in men (Ford and Liu, 2001). A reduction on HDL-C concentration of this magnitude was observed in three of the twelve subjects in this study.

Several previous studies have indicated that increased sugar intake may increase TAG level during low fat and high carbohydrate diets. Hudgins *et al.* (1996) Showed that change in the ratio of simple sugar to complex carbohydrate from 60:40 to 40:60 in low fat and high carbohydrate diet prevented stimulation of *de novo* lipogenesis. and an increase in energy from simple sugars was adversely associated with changes in HDL cholesterol concentrations among 55 overweight postmenopausal women (Bunyard *et al.* 2002). Although glucose is the monomer of the starch structure, it may physiologically act differently in human metabolism when included in an *ad libitum* intake of low-fat and high starch diet (Marckmann *et al.* 2000). This may be related to the GI of the food. Moreover much of the sugar in the diet may be from

fructose in the fruits and sucrose and high fructose corn syrup which has also been related to increased TAG (Gross *et al.* 2004). The current recommendation is that complex carbohydrates should provide more than 30% of daily energy intake and energy from simple sugar intake should reduce as much as possible. However this may be difficult for the general public to achieve if they do not understand the sugar contents of foods.

The GL of the diets of the women in this study increased and this may have adversely affected their plasma lipids. Results of an observational study of 280 apparently healthy postmenopausal women revealed that dietary GL and average GI were inversely related to plasma HDL concentrations and positively related to fasting plasma TAG levels, independent of BMI, weight change, total energy intake, and other known CHD risk factors (Liu *et al.* 2001).

Reduction in HDL cholesterol level is associated with increased risk of CHD. For every decrease of about 0.026 mmol/L in HDL-C concentration, the risk of CHD increases by 3.2% in women and 2.3% in men (Ford and Liu, 2001). After following the dietary intervention in this study, plasma HDL cholesterol concentrations decreased 4.5% and 9.8% after one and four weeks of dietary intervention, respectively. The consumption of a low fat diet results in more rapid clearance of HDL and decreased transport of HDL apo-proteins. However, it has been shown that diet-induced lowering HDL cholesterol level is not equal to reduced atherogenicity in some people consuming a high-fat diet (Brinton *et al.* 1990).

Ullman *et al.* (1991) showed that the gradual introduction of a high-carbohydrate diet prevented the hypertriacylglycerolaemic effect that occurred when a high-carbohydrate diet was introduced abruptly.

There is substantial evidence that in free-living situations low-fat high-carbohydrate diets lead to weight loss (Kasim-karakas *et al.* 1993; Leenen *et al.* 1993) that in turn may correct insulin resistance and plasma TAG metabolism (Purnell and Brunzell, 1997). In the present study, over the four weeks of dietary intervention, there was significant reduction in BMI and although there was a trend for increase in HOMA score it was not statistically significant.

It seems clear that the increase in TAG that was observed in this study was most likely to be due to an increase in carbohydrate intake, as this effect is well known and has been recognised for several decades. However, it is less clear whether it was the reduction in subjects' body weight and/or the dietary changes that caused the observed reduction in HDL cholesterol. In a study carried out by Kasim-Karakas *et al.* (2000) on 64 healthy postmenopausal women, the effects on HDL cholesterol of following an energy-controlled high-carbohydrate, low fat (HC-LF) diet for four months and an ad libitum HC-LF diet for eight months was studied. The authors reported that HDL cholesterol decreased on the energy controlled HC-LF diet in which no weight loss was observed and therefore the changes were thought to be due to dietary changes. However, HDL cholesterol remained low on the ad libitum HC-LF diet which suggests that weight loss can also contribute to lowering HDL cholesterol.

One very positive aspect of the dietary intervention in this study was that after following the recommended diet there was a significant increase in the intake of fruit and vegetables. This had the added benefit of increasing the antioxidant power of the subjects' plasma as measured by FRAP (Table 3.7 and 3.9). This appears to have mostly been associated with an increase in fruit intake (Table 3.9).

CRP has been introduced as an independent risk factor for type 2 diabetes and CHD (Ridker *et al.* 1999 and Ridker *et al* 2000). CRP is an acute phase inflammatory protein, and proven as a strong indicator of CHD risk (Ridker *et al.* 2001). The possible effects of the dietary changes on CRP level were examined, but there was no statistically significant change. In other studies, CRP was positively correlated with dietary GI (Liu *et al.* 2001). Liu *et al.* (2001) showed positive association between CRP concentration and dietary GI in large population. Healthy diet and exercise together were shown recently to reduce CRP concentration by 45% (Wegge *et al.* 2004). This study had a relatively small number of subjects compared to these studies that have detected changes in CRP and it is likely that it was not sufficiently powered to detect any change.

In conclusion, following the UK dietary guidelines by increasing dietary carbohydrate with emphasis on starch intake in postmenopausal women resulted in weight reduction and an increase in antioxidant power of plasma which should benefit health. However, the changes in lipid profile were more likely to favour an increased risk of CHD. There seem to be worse effects when women increased their simple sugar intake at the expense of starch. GI or GL were not used in the dietary advice in this study and this may have affected the interpretation of the guidelines.

The effects of these parameters are explored more in the following chapters particularly in the intervention study described in chapter 5.

As women who follow dietary guidelines in a free-living situation, rather than under very controlled conditions, are likely to make dietary changes in the manner of the women in this study, more research is needed to devise better and more appropriate guidelines for reducing CHD risk.

Chapter 4

Relationships between dietary glycaemic index and metabolic parameters in offspring of patients with type 2 diabetes and control subjects

4.1 Introduction

Type 2 diabetes, formerly known as non-insulin dependent diabetes or adult onset diabetes, is the most common form of diabetes and is estimated to affect more than 150 million adults worldwide (FAO/WHO, 2003). The disease is becoming more prevalent and the incidence is expected to double over the next 25 years (King *et al.* 1998). This is a worrying trend, as not only is it affecting a large proportion of the world's population, but it has also started to appear earlier in life and is now being identified in younger age groups including adolescents and children (Aboderin, 2001).

In recent years there has been a lot of interest in a condition called the metabolic syndrome, which was the term put forward by Reaven (1988) to describe a cluster of disorders linked with obesity and hyperinsulinaemia, and associated with increased risk of type 2 diabetes and CVD. Although a number of different sets of diagnostic criteria have been proposed for the metabolic syndrome (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001; European Group for the Study of Insulin Resistance, 2002), insulin resistance, hyperglycaemia, dyslipidaemia, central obesity and hypertension are generally agreed to be the five key features.

Insulin resistance is believed to occur up to ten or twenty years before type 2 diabetes develops (Wareham *et al.* 1990; Martin *et al.* 1992). Insulin resistance is the resistance of the tissues of the body to the effects of glucose. The body then has to produce more insulin to get the same action in response to normal blood glucose levels after a meal. This is thought to lead to gradually increasing glucose

concentrations, which result in even higher insulin secretion and eventually to the beta cells in the pancreas that produce insulin not working (Pan *et al.* 1997).

There are a number of complications associated with type 2 diabetes including increased risk of infections, coronary heart disease (CHD) and stroke (King *et al.* 1998; (FAO WHO, 2003)). In fact, individuals who develop type 2 diabetes are reported to have a three- to four fold higher risk of mortality and morbidity from CHD compared with healthy individuals (Niskanen *et al.* 1998).

Genetic and environmental factors are believed to influence the development of type 2 diabetes, and the increasing rates of the condition show the importance of environmental and lifestyle factors. The increasing rates of overweight and obesity, reductions in physical activity levels and changes in diet are thought to play particularly important roles in the increase in rates of type 2 diabetes. In many populations, it has been reported that being overweight or obese is associated with increased risk of type 2 diabetes, and this has especially been shown to be the case when too much of the excess adipose tissue is stored around the abdomen (FAO/WHO, 2003).

It is also known that having a genetic susceptibility to type 2 diabetes increases the risk of developing the condition. For example, certain ethnic groups such as the Pima Indians of Arizona in the USA show the highest known prevalence of type 2 diabetes of any population (Haffner, 1998; Lindsay *et al.* 2002). Furthermore, having a family history of type 2 diabetes increases one's risk of developing the condition, in fact, it has been reported that first degree relatives of patients with type 2 diabetes have a

three- to four fold higher risk of developing type 2 diabetes compared with those without this family background (Kobberling and Tillil, 1982).

While lifestyle factors, which contribute to the development of overweight, are certain to increase the risk of type 2 diabetes, whether dietary factors have an effect independent of this is not known.

However, a number of large-scale epidemiological studies have provided evidence for a link between habitual diet and the development of type 2 diabetes. A diet high in saturated fatty acids has been associated with higher fasting plasma glucose and insulin concentrations (Feskens *et al.* 1990; Parker *et al.* 1993), impaired glucose tolerance (Feskens *et al.* 1995), and higher proportions of saturated fatty acids in serum lipids and muscle phospholipids have been associated with greater risk of developing type 2 diabetes (Vessby *et al.* 1994). In contrast, there is evidence that a higher proportion of long-chain polyunsaturated fatty acids in skeletal muscle phospholipids is associated with increased insulin sensitivity (Salmeron *et al.* 2001), and higher dietary intake of unsaturated fatty acids has been associated with lower risk of developing type 2 diabetes (Meyer *et al.* 2001; Salmeron *et al.* 2001).

There is also evidence that dietary carbohydrates may influence the risk of developing type 2 diabetes, and a number of studies have reported that a lower dietary fibre intake is associated with an increased risk of developing type 2 diabetes (Salmeron *et al.* 1997a; Salmeron *et al.* 1997b; Meyer *et al.* 2000) while quite a few studies have reported no association between total carbohydrate intake and diabetes risk (Lundgren *et al.* 1989). Several studies have used the concept of GI in studying

relationships between dietary carbohydrate and the risk of type 2 diabetes. In the Nurses' Health Study, the multivariate-adjusted relative risk of type 2 diabetes during 6 years of follow-up was 1.37 (95% CI: 1.09, 1.71) for an increase in GI of 15 units and was 1.47 (95% CI: 1.16, 1.86) for extreme quintiles of dietary GL. Women with both a high dietary GL and a low cereal fibre intake were at an even higher risk of type 2 diabetes (relative risk: 2.43; 95% CI: 1.12, 5.27) (Salmeron *et al.* 1997a). In the Health Professional's Follow-up Study, the multivariate-adjusted relative risk was 1.37 (95% CI; 1.02, 1.83) in a 6-year follow-up for extreme quintiles of dietary GL and 2.17 (95% CI: 1.04, 4.54) for the combination of a high GL and a low intake of cereal fibre (Salmeron *et al.* 1997b). By contrast, no meaningful associations were found between GI and GL and diabetes risk in the 6 year follow up of the Iowa Women's Health Study cohort (Meyer *et al.* 2000).

A number of cross-sectional studies have also reported relationships between dietary GI and GL and metabolic risk factors for type 2 diabetes and CHD. In a cross-sectional study of 1,420 healthy adults in the UK, GI of the habitual diet was the only dietary variable found to be significantly related to serum HDL cholesterol concentrations (Frost *et al.* 1999). In the US, high dietary GL was associated with low plasma HDL concentrations and elevated fasting triacylglycerol concentrations in 280 postmenopausal women taking parting the Nurses' Health Study (Liu *et al.* 2001). In another cross-sectional analysis of a random sample of 244 healthy middle-aged women from the Women's Health Study (Liu *et al.* 2002), dietary GI was found to be significantly and positively associated with C-reactive protein concentration, which is reported to be a sensitive marker of systematic inflammation, independent of usual risk factors.

There have been a number of large-scale intervention studies reporting that changes in lifestyle including weight loss, increases in physical activity and dietary changes can reduce the risk of type 2 diabetes. In the Finnish Diabetes Prevention Study (Tuomilehto *et al.* 2001;Uusitupa *et al.* 2000), the incidence of type 2 diabetes was reduced by 58% following combined dietary and physical activity advice in 522 middle aged, overweight subjects with impaired glucose tolerance after a 3.2 year follow-up. Similarly, in the US Diabetes Prevention Study (Knowler *et al.* 2002), on 3,234 non-diabetic, high-risk individuals, a lifestyle intervention including advice to lose weight, to follow a 'healthy, low caloric, low fat diet' and to increase physical activity reduced the incidence of type 2 diabetes by 58% (95% CI: 48, 66%), and to a significantly greater extent compared with a control or intervention with a drug. In these studies, it is difficult to separate an effect of diet from other lifestyle advice, however, in the Da Qing Impared Glucose Tolerance Test and Diabetes Study (Pan *et al.* 1997), the influence of a dietary intervention was investigated separately. In this large-scale intervention study, involving 577 high-risk individuals, diet only, exercise only, and diet and exercise interventions, were found to significantly reduce the incidence of type 2 diabetes by 31%, 46% and 42%, respectively, over a 6-year follow-up period.

Thus, is seems to be the case that there is evidence from epidemiological, large-scale intervention and cross-sectional studies that diet may influence the development of type 2 diabetes. As I said at the start of this chapter, insulin resistance is a central metabolic problem in type 2 diabetes, and a number of possible mechanisms have been put forward that could explain how diet could influence insulin sensitivity.

Diets lower in fat might help people lose weight and reduce obesity which could improve glucose control in the body and insulin sensitivity (Hu *et al.* 2001). Alternatively, the effects of dietary fats and fatty acids could be explained by the their influence on the fatty acid composition of cell membranes in the body. It has been shown that increasing the proportion of polyunsaturated fatty acids and reducing the proportion of saturated fatty acids in phospholipids in skeletal muscle could improve insulin sensitivity (Borkman *et al.* 1993) by several mechanisms including altering insulin receptor binding or affinity and influencing ion permeability and cell signalling (Vessby, 2000). Furthermore, diets that are higher in unrefined carbohydrates and dietary fibre produce slower glycaemic and insulinaemic responses compared with more processed refined cereal grains and could protect against the development of type 2 diabetes in this way (Hu *et al.* 2001).

As I already mentioned, offspring of individuals with type 2 diabetes have a higher risk of developing this condition compared with people who have no family members with the disease (Kobberling *et al.* 1985), and this is thought to be due to genetic factors and also lifestyle factors. People in the same family have many similar lifestyle habits such as eating similar foods and cooking foods in the same way, frying versus grilling of foods. Indeed, studies that have looked into this have reported that the children's food intake and eating patterns are influenced by their parents' (Wardle, 1995; Feunekes *et al.* 1997). It has also been shown by a study, that the what people eat when they are adults is very close to what they ate when they were children and adolescents (Welten *et al.* 1997). Thus it could be believed that 'poor dietary habits' in families could influence or add to the higher risk of diabetes in their offspring. This idea is supported by a study carried out by Adamson

et al. (2001) who assessed the dietary intake of 149 non-diabetic relatives and the same number of age- and sex-matched control subjects with no family history of diabetes. These authors reported that relatives consumed diets that were more likely to promote rather than prevent the development of type 2 diabetes as their diets were higher in total fat, saturated fat and cholesterol and lower in carbohydrate and non-starch polysaccharide compared with control subjects. However, the researchers who carried out this study did not examine the dietary GI or GL of type 2 diabetic relatives.

Whether the GI or the GL of the habitual diet of high risk individuals such as type 2 diabetic offspring could increase the risk of developing the condition is not known, as I could find no other study that reported on this. Furthermore, to my knowledge, there is no published studies examining the relationships between habitual GI and GL, and anthropometric and metabolic risk factors that are known to be important for diabetes risk in this vulnerable group.

4.2 Objectives

The objectives of the study were:

- To assess habitual dietary intake, GI, GL and the metabolic risk factors for CHD and type 2 diabetes,
- To examine the relationships between habitual dietary intake, GI, GL and metabolic risk factors in offspring of patients with type 2 diabetes and in control subjects.

4.3 Materials and methods

The data used in this study was collected as part of a larger study (Higgins *et al.* 2004). It was my idea to look at GI in this study. I carried out the GI and GL calculations of the subjects' habitual diets and the comparisons and statistical analysis described in this chapter.

4.3.1. Subjects

Thirty-four healthy volunteers participated in this study; 17 (13 females and 4 males) were adult offspring of patients with type 2 diabetes (offspring group) and 17 were control subjects (control group). Subjects were individually matched for age (± 2 years) and gender. Subjects were not matched for fatness or adiposity as a number of previous studies (Humphriss *et al.* 1997; Ezenwaka *et al.* 2001; van Dam *et al.* 2001) have reported that offspring of patients with type 2 diabetes have greater levels of adiposity compared with individuals with no family history of diabetes. To take part in the study, subjects in the offspring group were required to have at least one parent diagnosed with type 2 diabetes before the age of 65 years and control subjects were required to have no first or second-degree relatives with type 2 diabetes. In addition, subjects were required to be; aged between 20 and 50 years, to have a fasting glucose less than 7mmol.l^{-1}, be healthy, pre-menopausal, not taking medications that affect lipid or carbohydrate metabolism (apart from oral contraceptives), not pregnant, not be on any special diet or taking any nutritional supplements. Three offspring subjects and five subjects in the control group were smokers. Ten and 11 of the subjects in the offspring and control groups, respectively were taking oral contraceptives at the time of study. The subjects were all of Northern European extraction. Ethical approval

was obtained from the Ethics committee at Glasgow Royal Infirmary and subjects provided written informed consent.

Subjects were recruited through advertisements in the University of Glasgow newsletter and by poster. Letters were also sent to patients with type 2 diabetes asking them if their adult daughters or sons would like to participate in the study. Subjects were mostly university staff, and postgraduate students and nurses.

4.3.2 Experimental Design

Subjects were required to attend a preliminary screening session at the Department of Human Nutrition at Yorkhill hospital in the fasted state. The subject information sheet was posted to interested volunteers in advance to allow them time to read it carefully and at the preliminary session, subjects were required to sign the consent form. At this visit, inclusion criteria were checked, anthropometric measurements were made, subjects also completed a health history questionnaire, and provided their contact details. Subjects were provided with a 7 day physical activity diary (the data on this is not presented here) and detailed instructions on how to complete it. Subjects were also provided with a 7-day diet diary, a digital food scales and detailed written and oral instruction on recording their habitual diet. Subjects were asked to keep a record of their usual diet for 7 days prior to the subsequent visit. For the second visit, subjects were collected in a taxi, after an overnight fast, and brought to the study room at the Department of Human Nutrition at Yorkhill or at Glasgow Royal Infirmary Hospitals, depending on which site was more convenient to the subject. Subjects were required to abstain from alcohol and vigorous physical activity for 24 hours before this visit. At this visit, subjects were required to take part

in an oral glucose tolerance test. Subjects also had their completed diet and physical activity diaries checked at this visit.

4.3.3 Anthropometric measurements

Height (m) (Holtain Ltd, Crymych, Dyfed), weight (kg) (SECA scales), waist circumference (cm), hip circumference (cm) and mid-upper arm circumference (cm) (MUAC) were measured. Body mass index (BMI) was calculated from weight and height (weight (kg)/ height (m^2)). Body fat was estimated by skinfold thickness. The skinfold measurements were made using calipers (Holtain Ltd. Crymych, UK) which measure the thickness of a fold of skin with its underlying layer of fat which were measured at four different sites; biceps; triceps; subscapular; suprailliac. Three measurements were taken at each site and averaged, and the sum of the four values was used to calculate the % body fat of each subject using the equations of Durnin & Womersley (1974).

4.3.4 Dietary assessments

Subjects were asked to keep a detailed record of their habitual dietary intake for 7 days (Bingham, 1987) immediately prior to visiting the laboratory for blood sampling. Subjects were provided with portable electronic food scales (Slater Household Ltd, Tonbridge, UK), a food dairy, and oral and written instructions on how to record their diets, and on correct use of the scales. The completed diet records were inspected on their return to ensure that they were complete, and that sufficient detail had been recorded. Subjects were asked keep a record of all foods and drinks consumed and asked to weigh each food item immediately prior to consumption. The diet records were analysed for daily energy and nutrient intakes using a computerised

version (Diet 5™, Robert Gordon University, Aberdeen) of the food composition tables (Holland *et al.* 1991).

Subjects' diet records were not included in the statistical analysis if their reported energy intakes were less than their estimated basal metabolic rate (BMR) multiplied by 1.1 (under-reporters) or greater than 2.0 (over-reporters). BMR was estimated using the Scofield equations (Schofield *et al.* 1985). These cut-offs were used as it is highly unlikely that habitual energy intake would be < 1.1 × BMR or > 2.0 × BMR (Goldberg *et al.* 1991). On this basis, one subject was excluded for under-reporting. This will explain which there are 17 subjects in the offspring group but only 16 subjects in the control group in the dietary results tables.

4.3.5 Calculation of glycaemic index (GI) and glycemic load (GL)

Subjects kept detailed weighed records of their habitual dietary intakes for seven days, which in addition to being analysed for daily energy and nutrient intakes, were also used for the estimation of GI and GL. Firstly, the GL of each carbohydrate-containing food recorded in the diet diary was estimated by multiplying the carbohydrate content of the serving of food (obtained from Diet 5) by the GI of that food item (obtained from the International Table of Glycaemic Index and Glycaemic Load; Foster-Powell *et al.* 2002) divided by 100 (Brand-Miller *et al.* 2003). The GL values, calculated in this way, for each carbohydrate-containing food consumed was summed to give the GL for seven days, and this value was then divided by seven to give the daily GL. The daily GI value was then calculated by dividing the daily GL by the total daily carbohydrate intake (obtained from Diet 5), and by multiplying by 100 (Foster-Powell *et al.* 2002).

4.3.6 Oral glucose tolerance test (OGTT)

Subjects arrived at the laboratory after an overnight fast (12 h) and were asked to refrain from alcohol and vigorous physical activity for 24 h before this visit. On arrival, each subject had a cannula placed in the ante-cubital fossa vein in the non-dominant arm. Blood samples were taken before and 15, 30, 60, 90, and 120 minutes after subjects consumed a 75 g oral glucose load (75g glucose dissolved in 250 ml water). Sterile saline (9g/l NaCl, 5 ml) was used to prevent blood from clotting in the cannula during the test period. The samples were immediately centrifuged for 10 min at 3,000 rpm at 4°C and plasma separated and stored at –80°C.

4.3.7 Laboratory analysis

Blood samples were collected into ethylenediaminetetra acetic (EDTA), sodium fluoride and lithium heparin vacutainers after a 12-hour overnight fast and immediately placed on wet ice. Plasma was separated by low speed centrifugation (MSE Leicester, U.K) at 3000rpm for 10 minutes at 4°C. Plasma was divided into aliquots and stored at -70°C until analysis.

All metabolic parameters were measured either at the Department of Pathological Biochemistry or the Department of Biochemistry at the Glasgow Royal Infirmary and full details of each of the methods are given in the methodology chapter (Chapter 2). I will briefly describe the method here. Plasma samples collected into EDTA were used for the determination of triaclyglycerol and total and high-density lipoprotein (HDL) cholesterol by standard enzymatic colorimetric procedures (Roche Diagnostic Corporation, Lewes, UK). Low-density lipoprotein (LDL) cholesterol

concentrations were calculated using the Friedelwald equation (Friedelwald *et al.* 1972). Non-esterified fatty acid (NEFA) concentrations were determined in EDTA plasma using a standard enzymatic method (Roche Diagnostic Corporation, Lewes, UK) Plasma samples collected into sodium fluoride were analysed for glucose concentrations using a standard enzymatic method (Roche Diagnostic Corporation, Lewes, UK). Insulin concentrations were determined in lithium heparin plasma by the Department of Biochemistry. Analysis of C-reactive protein (CRP) was carried out in EDTA plasma samples using an in house ELISA (Highton and Hessian, 1984). Adiponectin concentrations were also measured in EDTA plasma samples using a commercially available ELISA (B-Bridge International, Inc. Japan).

The homeostatic model assessment ($HOMA_{IR}$) technique (fasting insulin x fasting glucose/22.5) was used as a measure of insulin resistance (Matthews *et al.* 1985). Additionally, insulin sensitivity was estimated using fasting and oral glucose tolerance test glucose, insulin and NEFA concentrations using the Belfiore *et al.* (2001) method which has been validated against the euglycemic clamp technique.

4.3.8 Statistical analysis

Data were expressed as mean ± standard deviation (SD). Data was checked for normality by visual inspection of the histograms in SPSS. Anthropometric data was found to be normally distributed but the dietary and metabolic parameter data were found not to be normally distributed. Where data was normally distributed, the student's independent t-test was used to compare mean values between the groups and to test for significant differences. Where data was not normally distributed, differences between the groups were assessed using the non-parametric, Mann

Whitney U test. In order to explore relationships between the variables, the non-parametric Spearman's correlation coefficient test was used. Chi Square and Fisher Exact tests (when required) were used for examining associations between categorical variables. Statistical analysis was performed using SPSS (version 11.0) and P values were accepted as being statistically significant when they were less than 0.05.

4.4 Results

4.4.1 Anthropometric Characteristics

Subjects were matched for gender and age (± 2 years), and therefore, no significant difference in the age of the groups was found or expected. There were no significant differences in any of the anthropometric measurements between the offspring and control groups (Table 4.1)

Table 4.1. Anthropometric Characteristics

	Offspring n=17		Control n=17		P*
	Mean	**SD**	**Mean**	**SD**	
Age (years)	32.7	7.1	35.1	6.0	0.302
Weight (kg)	70.6	9.6	66.8	10.0	0.809
Height (m)	1.7	0.1	1.7	0.1	0.270
Body mass index (kg.m^{-2})	25.8	3.3	24.1	3.1	0.137
Body fat (%)	29.8	5.9	27.6	5.9	0.278
Waist circumference (cm)	81.8	9.0	77.7	9.8	0.217
Waist to hip ratio	0.8	0.1	0.8	0.1	0.404
Mid upper arm circumference (cm)	30.6	2.4	29.0	3.1	0.122

*** Independent Student t-test**

The majority (64.7%) of offspring and 35.3% of control subjects were either overweight or obese (BMI ≥ 25 kg.m^{-2}) (P=0.086) (WHO 1998). A significantly higher proportion of subjects in the offspring group had a BMI > 27.5 kg.m^{-2} (35.3 %) compared with the control group (5.9 %) (P = 0.043, for one-sided Fisher exact test) (Table 4.2). This cut-off of 27 kg.m^{-2} was suggested to be used by Sargeant *et al.* (2000). In addition, the prevalence of high waist circumferences (>80 cm for women and >94 cm for men) (Department of Health 2004) was 17.6% in both

groups. No difference in the prevalence of high waist to hip ratio (0.85 or greater for women and 0.95 or greater in men) (Department of Health, 2004) was observed between the two groups (the prevalence was three of 17 subjects in both groups).

Table 4.2 Proportion of the offspring and control groups with BMI less than and greater than 25 and 27.5 $kg.m^{-2}$.

BMI ($kg.m^{-2}$)	Offspring (n=17) n (%)	Control (n=17) n (%)	P
<25	6 (35.3)	11 (64.7)	0.086
(Overweight or obese)	11 (64.7)	6 (35.3)	
≤27.5	11 (64.7)	16 (94.1)	**0.043**
>27.5	6 (35.3)	1 (5.9)	

When the anthropometric characterisitics for the subjects were analysed separately, female offspring had a significantly higher waist to hip ratio than control subjects (P = 0.036) (Table 4.3). Waist circumference and body mass index were also higher in female offspring compared with female control subjects, however, the differences did not quite reach statistical significance (P=0.063 and P=0.083, respectively). There were no other significant differences in any of the other anthropomtric variables between female or male offspring and control subjects (Table 4.3).

Table 4.3. Subject characteristics of the offspring and control groups by gender

	Females					Males				
	Offspring *n*=13		Control *n*=13		P*	Offspring *n*=4		Control *n*=4		*P**
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
Age (years)	33.2	7.0	35.8	5.6	0.319	31.0	7.9	32.7	7.4	0.757
Weight (kg)	68.4	8.4	63.9	8.1	0.174	77.9	11.1	76.5	10.4	0.862
Height (m)	1.6	0.04	1.6	0.1	0.376	1.8	0.1	1.8	0.05	0.563
Body mass index ($kg.m^{-2}$)	26.2	3.0	24.0	3.3	**0.083**	24.4	4.2	24.5	2.7	0.948
Body fat (%)	32.1	3.6	30.3	2.5	0.156	22.5	6.5	18.8	5.0	0.403
Waist circumference (cm)	80.5	8.9	74.5	6.7	**0.063**	85.9	9.2	88.2	11.8	0.761
Waist to hip ratio	0.8	0.05	0.8	0.04	**0.036**	0.8	0.1	0.9	0.1	0.470
Mid upper arm circumference (cm)	30.5	2.4	28.8	3.2	0.171	31.0	3.0	29.5	3.0	0.544

*** Independent Student t-test**

Table 4.4. Daily energy and macronutrient intakes in the offspring and control subjects by weighed diet record

	Offspring *n*=17		Control *n*=16		*P**
	Mean	SD	Mean	SD	
Energy (kcal)	2,124	401	2,112	453	0.857
Energy (kJ)	8,887	1,678	8,837	1,895	0.857
Nutrient (g)					
Total fat	73.2	21.7	72.9	11.9	1.00
Saturated fat	27.3	10.4	25.2	4.7	0.627
Monounsaturated fat	22.6	7.2	24.2	4.5	0.439
Polyunsaturated fat	12.6	4.3	13.3	4.3	0.639
Carbohydrate	272.9	60.6	260.1	64.5	0.564
Sugar	105.3	32.4	102.9	36.0	0.517
Starch	148.4	40.7	125.3	22.5	**0.044**
Non-milk extrinsic sugar	72.4	30.6	66.4	26.8	0.540
Non-starch polysaccharide	15.1	3.5	14.6	6.2	0.705
Protein	85.7	16.0	79.1	21.1	0.078
Alcohol	14.1	9.0	22.2	18.1	0.407

*** Mann Whitney U test**

4.4.2 Dietary Intake

There were no significant differences in daily energy or in absolute amounts of macronutrients between offspring and control subjects with the exception of starch intakes, which were significantly higher in offspring compared with control subjects (P=0.044) (Table 4.4). GI and GL values were not significantly different between the groups (Table 4.5).

Table 4.5 - Dietary glycaemic index and glycaemic load in the offspring and control subjects by weighed diet record

	Offspring (n=17)		Control (n=16)		*P**
	Mean	**SD**	**Mean**	**SD**	
Glycaemic load	147.0	34.0	140.7	40.5	0.460
Glycaemic index	54.0	2.8	54.0	4.9	0.971

*** Mann Whitney U test**

Table 4.6. Percentage of energy intake from macronutrients in the offspring and control subjects by weighed diet record

	Offspring (n=17)		Control (n=16)		P*
	Mean	**SD**	**Mean**	**SD**	
% energy from:					
Total fat	30.3	5.6	31.0	4.3	0.601
Saturated fat	11.2	3.0	10.7	1.9	0.614
Monounsaturated fat	9.5	2.3	10.2	1.5	0.482
Polyunsaturated fat	5.1	1.5	5.6	1.7	0.470
Carbohydrate	48.0	6.5	46.8	5.9	0.418
Sugar	19.1	5.4	18.5	4.1	0.540
Starch	28.1	6.2	24.2	4.2	**0.023**
Non-milk extrinsic sugar	12.8	4.3	12.0	4.2	0.692
Protein	16.6	3.3	15.2	2.3	0.227
Alcohol	4.5	2.8	6.3	4.6	0.339

*** Mann Whitney U test**

Apart from the percentage of energy from starch, which was significantly higher (P = 0.023) in the offspring group, there were no significant differences in the percentage of energy from carbohydrate, fat, protein or alcohol between the groups (Table 4.6).

Table 4.7. The proportion of the offspring and control subjects meeting the UK dietary targets (Scottish Office, 1993)

Nutrient intake	Dietary target	Offspring n=17	Control n=16	P*
% Energy from				
Carbohydrate	≥ 47	58.8	43.7	0.387
Non-milk extrinsic sugar	< 11	35.3	43.8	0.619
Starch	≥ 37	0	0	-
Sugar: starch ratio	<0.66	35.3	23.5	0.452
Non-starch polysaccharides (g)	≥ 18	23.5	12.5	0.656
% Energy from				
Total fat	< 33	76.5	56.3	0.218
Saturated fat	< 10	35.3	37.5	0.895
Monounsaturated fat	< 12	88.2	87.5	1.000
Polyunsaturated fat	< 6	88.2	68.8	0.225
Alcohol	< 5	58.8	50.0	0.611

* **Chi square test**

The proportion of subjects meeting the UK dietary targets (Scottish Office, 1993) was not significantly different between the two groups. However, a low proportion of subjects from both groups achieved the dietary targets, and this was especially so for percentage energy from non-milk extrinsic sugars, starch, NSP and saturated fat, showing that both groups consumed diets that were too high in non-milk extrinsic sugars and saturated fat and too low in starch and NSP (Table 4.7).

Table 4.8. Metabolic parameters in offspring and control subjects

Biochemical factors	Offspring *n*=17		Control *n*=17		*P**
	Mean	SD	Mean	SD	
Total cholesterol ($mmol.l^{-1}$)	4.31	0.83	4.54	0.67	0.367
Triacylglycerol ($mmol.l^{-1}$)	1.38	1.21	1.01	0.35	0.245
VLDL cholesterol ($mmol.l^{-1}$)	0.45	0.50	0.25	0.12	0.116
LDL cholesterol ($mmol.l^{-1}$)	2.59	0.72	2.69	0.85	0.710
HDL cholesterol ($mmol.l^{-1}$)	1.27	0.20	1.61	0.45	**0.011**
LDL_3 ($mmol.l^{-1}$)	1.32	1.53	1.09	1.07	0.804
Fasting glucose ($mmol.l^{-1}$)	5.16	0.59	5.20	0.45	0.844
2 hour glucose ($mmol.l^{-1}$)	6.75	2.20	5.69	1.41	0.121
Fasting insulin ($\mu U.ml^{-1}$)	8.12	5.86	5.02	1.58	**0.049**
C-reactive protein ($mg.l^{-1}$)	2.23	2.66	1.37	1.73	0.551
Adiponectin ($mg.l^{-1}$)	6.98	3.65	8.16	4.61	0.278
Fasting NEFA ($mmol.l^{-1}$)	0.48	0.20	0.51	0.19	0.697
$HOMA_{IR}$	1.97	1.56	1.16	0.37	**0.052**
Insulin Sensitivity Index (Glu)	0.84	0.62	0.92	0.54	0.241
Insulin Sensitivity Index (FFA)	1.20	0.77	1.11	0.73	0.478

*** Mann Whitney U test**

4.4.3 Metabolic Parameters

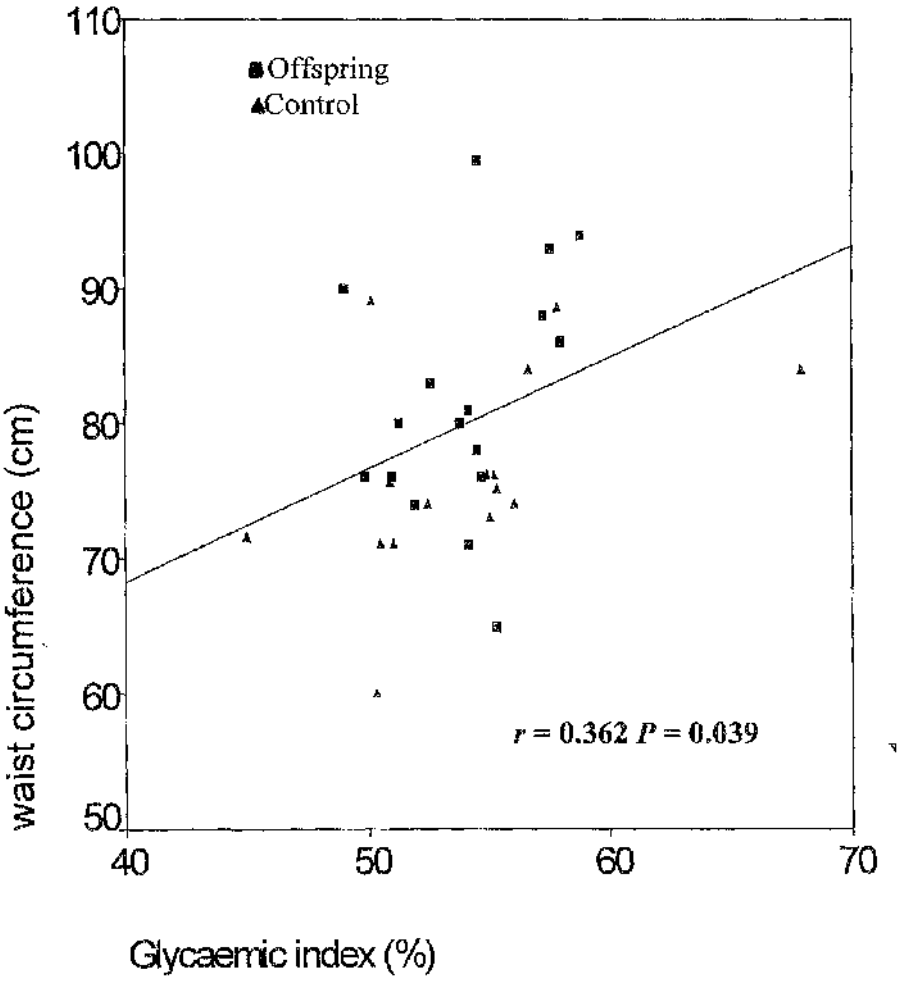
The results show that HDL cholesterol concentrations were significantly lower (P = 0.011) and fasting insulin concentrations were significantly higher (P = 0.049) in the offspring compared with control subjects. $HOMA_{IR}$ score was higher in offspring compared with control subjects but the difference did not quite reach to statistical significance (P = 0.052). There were no significant differences in any other metabolic parameters measured (Table 4.8).

As there were no significant differences in habitual dietary intake, GI or GL between offspring and control subjects, the data for both groups was combined to look at the relationships between the dietary parameters and anthropometric characteristics and metabolic risk factors. Figure 3.1 shows the relationship between dietary GI and waist circumference in the study subjects. Dietary GI was positively and significantly correlated with waist circumference, an indicator of central obesity. GI was also significantly correlated with waist: hip ratio (r = 0.43, P = 0.013) but not correlated with BMI or percentage body fat. GL was not significantly correlated with any of the anthropometric characteristics. Furthermore, neither dietary GI or GL were significantly correlated with any of the metabolic risk factors measured.

Table 4.9 Relationships between anthropometric characteristics and some metabolic risk factors (n = 34)

	Waist circumference (cm)		**BMI ($kg.m^{-2}$)**	
	r	***P***	***r***	***P***
Total cholesterol ($mmol.l^{-1}$)	**0.38**	**0.025**	0.33	0.054
Triacylglycerol ($mmol.l^{-1}$)	**0.63**	**0.001**	**0.49**	**0.003**
VLDL cholesterol ($mmol.l^{-1}$)	**0.38**	**0.020**	**0.42**	**0.014**
LDL cholesterol ($mmol.l^{-1}$)	**0.51**	**0.002**	**0.36**	**0.034**
HDL cholesterol ($mmol.l^{-1}$)	**-0.69**	**0.001**	**-0.49**	**0.003**
LDL_3 ($mmol.l^{-1}$)	**0.54**	**0.018**	0.01	0.977
TC:HDL cholesterol ratio	**0.72**	**0.001**	**0.58**	**0.001**
C-reactive protein ($mg.l^{-1}$)	0.21	0.241	**0.41**	**0.018**
Adiponectin ($mg.l^{-1}$)	**-0.36**	**0.044**	0.08	0.643
Fasting NEFA ($mmol.l^{-1}$)	0.12	0.510	0.01	0.597
$HOMA_{IR}$	**0.52**	**0.002**	**0.50**	**0.003**
Insulin sensitivity index (Glu)	**-0.39**	**0.043**	-0.34	0.074
Insulin sensitivity index (NEFA)	**-0.42**	**0.026**	**-0.53**	**0.004**

Figure 4.10. Relationship between dietary glycaemic index (%) and waist circumference (cm) (Spearman's Correlation Coefficient)



4.4.4 Relationships between anthropometric characteristics and metabolic parameters

Waist circumference ws positively correlated with fasting lipids, total cholesterol, triacylglycerol, VLDL and LDL cholesterol, total cholesterol to HDL cholesterol ratio, LDL_3, and $HOMA_{IR}$ score and negatively correlated with HDL cholesterol, HDL_2 and the insulin sensitivity indices (Table 4.9). BMI was positively correlated with TAG, VLDL and LDL cholesterol, total to HDL cholesterol ratio, CRP and $HOMA_{IR}$ score and was inversely correlated with the HDL cholesterol, and the insulin sensitivity index assess against NEFA (Table 4.9)

$HOMA_{IR}$ score was significantly and positively correlated with BMI, waist circumference and waist to hip ratio (Table 4.10), while the insulin sensitivity indices were inversely correlated with waist circumference, and the insulin sensitivity index assessed against NEFA was inversely correlated against BMI. $HOMA_{IR}$ was positively correlated with fasting triacylglycerol, LDL cholesterol, total to HDL cholesterol ratio and C-reactive protein and inversely correlated with HDL cholesterol concentrations. The insulin sensitivity index assessed against glucose was inversely correlated with VLDL cholesterol, C-reactive protein and positively correlated with adiponectin concentrations. The insulin sensitivity index assessed against NEFA was inversely correlated with fasting triaclyglycerol, VLDL cholesterol and C-reactive protein (Table 4.10).

Table 4.10 Relationships between $HOMA_{IR}$ and the insulin sensitivity indices (ISI GLU, ISI NEFA) with anthropometric characteristics and metabolic risk factors

	$HOMA_{IR}$		ISI (GLU)		ISI (NEFA)	
	r	P	r	P	R	P
BMI ($kg.m^{-2}$)	**0.46**	**0.007**	-0.34	0.074	**-0.53**	**0.004**
Waist (cm)	**0.52**	**0.002**	**-0.39**	**0.043**	**-0.42**	**0.026**
Waist to hip ratio	**0.49**	**0.003**	-0.36	0.057	-0.24	0.212
Total cholesterol ($mmol.l^{-1}$)	0.30	0.085	-0.15	0.448	-0.27	0.162
Triacylglycerol ($mmol.l^{-1}$)	**0.48**	**0.004**	-0.36	0.061	**-0.55**	**0.003**
VLDL cholesterol ($mmol.l^{-1}$)	0.22	0.220	**-0.37**	**0.050**	**-0.43**	**0.002**
LDL cholesterol ($mmol.l^{-1}$)	**0.36**	**0.038**	-0.11	0.569	-0.20	0.298
HDL cholesterol ($mmol.l^{-1}$)	**-0.35**	**0.041**	0.27	0.158	0.25	0.194
TC:HDL cholesterol ratio	**0.41**	**0.016**	-0.23	0.238	-0.31	0.103
Fasting NEFA ($mmol.l^{-1}$)	-0.14	0.458	0.07	0.716	-0.25	0.200
C-reactive protein ($mg.l^{-1}$)	**0.41**	**0.019**	**-0.58**	**0.002**	**-0.74**	**0.001**
Adiponectin ($mg.l^{-1}$)	-0.26	0.147	**0.53**	**0.004**	0.37	0.060

In offspring, CRP was positively correlated associated with BMI, waist circumference, $HOMA_{IR}$ score and fasting triaclyglycerol concentrations and inversely correlated with the insulin sensitivity indices. In the control group, CRP was only correlated with the insulin sensitivity indices (Table 4.11).

Table 4.11 Relationships between C-reactive protein with anthropometric characteristics and metabolic parameters in offspring and control groups separately

	Offspring (n=17)		Control (n=17)	
	R	P	r	P
BMI ($kg.m^{-2}$)	**0.65**	**0.005**	0.13	0.648
Waist circumference (cm)	**0.49**	**0.046**	-0.32	0.239
$HOMA_{IR}$	**0.59**	**0.013**	0.02	0.950
Insulin sensitivity index (Glu)	**-0.60**	**0.015**	**-0.57**	**0.066**
Insulin sensitivity index (NEFA)	**-0.77**	**0.001**	**-0.65**	**0.032**
Total cholesterol ($mmol.l^{-1}$)	0.39	0.126	-0.07	0.795
Triacylglycerol ($mmol.l^{-1}$)	**0.57**	**0.017**	-0.08	0.775
HDL cholesterol ($mmol.l^{-1}$)	-0.31	0.227	0.36	0.183
TC:HDL cholesterol ratio	0.36	0.154	-0.34	0.211
Adiponectin ($mg.l^{-1}$)	-0.26	0.309	0.11	0.694
Fasting NEFA ($mmol.l^{-1}$)	0.40	0.130	-0.01	0.974

4.5 Discussion

The objectives of the study were, first, to assess habitual dietary intake, glycaemic index (GI), glycaemic load (GL) and metabolic risk factors for CHD and type 2 diabetes, in offspring of patients with type 2 diabetes and in control subjects. Secondly, to examine the relationships between dietary GI and GL with anthropometric characteristics and metabolic risk factors in these subjects.

The main findings of the current study were that there were no differences in habitual dietary intake, GI or GL between offspring and control subjects. Offspring were found to have greater levels of adiposity with a greater proportion of the offspring subjects having a BMI > 27.5 $kg.m^2$. Female offspring were found to have a significantly higher waist to hip ratio ($P = 0.036$), and a higher waist circumference ($P = 0.063$) and BMI ($P = 0.083$) compared with female control subjects. Offspring were found to be significantly more insulin resistant compared with control subjects with significantly higher fasting insulin ($P = 0.049$) and higher $HOMA_{IR}$ ($P = 0.052$), and significantly lower HDL cholesterol concentrations ($P = 0.011$). While dietary GI and GL were not found to be directly associated with any of the metabolic parameters measured in the study, GI was positively correlated with waist circumference ($P = 0.039$) and waist to hip ratio ($P = 0.043$), and these measures of adiposity (i.e. waist circumference and BMI) were significantly correlated with many of the metabolic parameters measured in the study. Thus, while the glycaemic quality of the diet did not appear to directly influence metabolic risk factors, the results do support the idea that they influence metabolic risk factors through their affect on adiposity, and in particular central adiposity.

There was no difference in absolute daily energy or macronutrient intakes (Table 4.4) or in the proportion of energy from the main energy providing nutrients (Table 4.6) between offspring and control groups. This finding of no significant difference in dietary intake is in agreement with the results of a study carried out by Johanson *et al.* (2003) but in disagreement with the results of an earlier study by Adamson *et al.* (2001). In the present study, and in the study by Johanson *et al.* (2003), habitual dietary intake was assessed by 7-day weighed intake. In contrast, Adamson *et al.* (2001) used a food frequency questionnaire to assess habitual dietary intake, and the authors admitted excluding more than half of their subjects (60%) due to suspected unreliable reporting, suggesting that there may have been a problem with the questionnaire that they used. Adamson *et al.*'s (2001) findings of 'less healthy' habitual diet in relatives of patients with type 2 diabetes compared with control subjects led the authors to suggest that "shared dietary habits amongst families could contribute to increased familial diabetes risk". We found that dietary intake was similar between offspring and control subjects and the dietary assessment method that we used was a more reliable method. Thus, if our results are correct it might be the case that factors other than family influences on diet may be more important. This might not be that surprising since there are many factors that influence diet, including current trends and fashions in diet and cuisine, intensive advertising by the food industry and large supermarket chains, and also 'healthy eating' campaigns by health promotion groups (Food Standards Agency, 2004).

Dietary GI or GL were not found to be significantly different between offspring and control subjects, in the present study (Table 4.5.). However, the GI and GL values reported in the current study (GI 54 in both offspring and control subjects, GL 147 in

offspring and 141 in control subjects, respectively) are similar to values reported in healthy adult volunteers in the UK (Haji Faraji *et al.* 2003). To my knowledge, no other study has reported on the dietary GI or GL of type 2 diabetic offspring, thus, very little is known regarding these dietary parameters, which have been shown to be associated with diabetes risk (Salmeron *et al.* 1997a and b).

The proportion of subjects in both the offspring and control groups who were meeting the UK dietary reference values (Department of Health, 1991), were compared and were found not to be significantly different (Table 4.7). However, a relatively small proportion of subjects in both groups were found to be achieving the dietary targets, and this was especially so for the percentage energy from starch, non-milk extrinsic sugars (NMES), NSP and saturated fat, showing that both groups consumed diets that were too high in NMES and saturated fat and too low in starch and NSP, thus both groups should try to improve their diet. Furthermore, these dietary characteristics are associated with higher levels of insulin resistance, poorer glycaemic control and adverse effects on blood lipids (Hung *et al.* 2003).

It has been reported by a number of studies (Perseghin *et al.* 1997; Humphriss *et al.* 1997; Ezenwaka *et al.* 2001), that offspring of patients with type 2 diabetes have a metabolic profile compared with the general population. In agreement with these findings, the offspring in the present study appeared to have significantly more insulin resistance with significantly higher fasting insulin ($P = 0.049$), higher $HOMA_{IR}$ scores ($P = 0.052$) and significantly lower HDL cholesterol concentrations ($P = 0.011$). We also found that a greater proportion of offspring subjects had a BMI above the cut-off (BMI > 27.5) recommended by (Sargeant *et al.* 2001) and that female offspring had

higher BMI (P = 0.083) and a greater degree of abdominal obesity (waist to hip ratio P = 0.036; waist circumference P = 0.063) compared with female control subjects, this is also in agreement with other studies (Humphriss *et al.* 1997; Ezenwaka *et al.* 2001; Vaag *et al.* 2001). The offspring in this study were found to have many of the characteristics of the 'metabolic syndrome' (i.e. higher BMI and waist circumference, higher fasting insulin and $HOMA_{IR}$ and lower HDL cholesterol concentrations) (Gibney *et al.* 2005) compared with individuals with no family history of type 2 diabetes. This is not a new finding and has been reported before (Humphriss *et al.* 1997; Ezenwaka *et al.* 2001; Vaag *et al.* 2001).

As habitual dietary intake, GI and GL were found to be similar between offspring and control groups, I decided to combine the data for both groups, to examine the relationships between the dietary parameters and the anthropometric and metabolic characteristics measured in the study. Dietary GI was found to be positively correlated with waist circumference (r = 0.36; P = 0.039; Figure 4.1) and waist to hip ratio (r = 0.43; P = 0.013), in that as the as the GI of the diet increased, abdominal adiposity also increased. I was surprised that GL was not found to be significantly associated to any of the anthropometric characteristics.

Waist circumference, which is a measure of abdominal obesity, was found to be positively correlated with fasting lipids, total cholesterol, triacylglycerol, VLDL and LDL cholesterol, total cholesterol to HDL cholesterol ratio, LDL_3, and $HOMA_{IR}$ score and negatively correlated with HDL cholesterol, and the insulin sensitivity indices. BMI was positively correlated with triaclyglycerol, VLDL cholesterol, LDL cholesterol, total to HDL cholesterol ratio, C-reactive protein and $HOMA_{IR}$ and was

inversely correlated with HDL cholesterol concentrations and the insulin sensitivity index assessed against NEFA. Thus, a higher waist circumference and BMI were associated with a more adverse metabolic profile (Table 4.9). There are many different studies that have reported that increasing adiposity is associated with an increase in metabolic risk factors that increase risk of CHD and type 2 diabetes (Grundy, 2004).

Dietary GI and GL were not significantly correlated with any of the metabolic parameters measured in the study. However, GI was positively correlated with waist circumference, and waist circumference was significantly associated with most of the metabolic parameters. These correlations support the idea that the dietary GI could influence metabolic risk for diabetes through its effects on abdominal adiposity. Of course, correlations do not prove cause and effect. A randomised control trial would need to be set up to look at the effect of high and low GI diets on metabolic risk factors to confirm this idea.

In the present study, plasma CRP levels were not significantly different between offspring and control subjects (2.23 vs 1.37 $mg.l^{-1}$, *NS*; Table 4.8). My results do not agree with the results of a study by Pannacciulli *et al.* (2002) who carried out a study of 162 non-smoking women, and found that women with a family history of type 2 diabetes (n = 95) had significantly higher CRP plasma levels than age- and BMI-matched control subject (n = 67; 5.5 vs. 3.5 $mg.l^{-1}$, $P = 0.012$). However, our results of similar CRP plasma levels in offspring and control subjects are in agreement with a more recent report by Kriketos *et al.* (2004) who reported that first-degree relatives of patients with type 2 diabetes had normal and comparable levels of CRP. However,

like my study, the numbers in this study (19 first degree relatives and 22 control subjects) were relatively small, and the study may not have had adequate statistical power to detect a statistically significant difference between the two groups.

The relationships between plasma CRP levels and anthropometric and metabolic parameters were explored in this study (Table 4.11), and interestingly, it was found that in offspring, CRP was positively correlated with BMI, waist circumference, $HOMA_{IR}$ and fasting triaclyglycerol concentrations and inversely correlated with the insulin sensitivity indices, while in control subjects, CRP was only significantly correlated (inversely) with insulin sensitivity, suggesting the CRP is more sensitive to increasing adiposity in people with a family history of type 2 diabetes. Pannacciulli *et al.* (2002) also reported that CRP levels were positively correlated with BMI, waist circumference and $HOMA_{IR}$, in a pooled analysis of females with and without a family history of type 2 diabetes. Similarily, Yudkin *et al.* (1999) reported in a cross-sectional study of 107 non-diabetic subjects, that CRP levels were related to insulin resistance, blood pressure, HDL and fasting triaclyglycerol concentrations. Therefore, my results are in agreement with previous studies (Yudkin *et al.* 1999; Pannacciulli *et al.* 2001; 2002) which reported a positive association (possibly mediated by cytokines produced by adipose tissue) of BMI, waist and insulin resistance with plasma CRP concentrations.

In conclusion, in this study, habitual diet, GI or GL were not found to be different between offspring and control subjects. Both offspring and control subjects did not comply well with the current dietary guidelines and could have improved their diets. It is important for individuals, and especially for offspring of patients with type 2

diabetes to have a healthy diet as a number of recent large intervention studies (Pan *et al.* 1997; Uusitupa *et al.* 2000; Tuomilehto *et al.* 2001; Knowler *et al.* 2002) have shown that improving diet can substantially reduce risk of developing type 2 diabetes in people who have a high risk. Although my results showed that GI was associated with measures of abdominal adiposity, I did not find any significant correlations between GI and any of the metabolic risk factors. It is possible that GI could influence metabolic risk factors through an effect on abdominal adiposity, however, my study did not prove this. The reasons for this could have been that the study was cross-sectional rather than an intervention study, and also the study may not have had enough subjects to detect significant relationships. This does not mean that GI does not influence metabolic risk, as there are many epidemiological studies that have shown that GI may be important for risk of type 2 diabetes (Salmeron *et al.* 1997a; Salmeron *et al.* 1997b; Frost *et al.* 1999; Liu *et al.* 2000; Liu *et al.* 2001).

Chapter 5

The effect of high and low glycaemic index diets on metabolic risk factors for CHD

5.1 Introduction

Coronary heart disease (CHD) is a leading cause of mortality and morbidity worldwide (FAO/WHO, 2003), and is the most common cause of death in the United Kingdom, being responsible for 22% and 17% of deaths in males and females, respectively (British Heart Foundation, 2004). Type 2 diabetes is also a major public health concern and the number of people with the disease is expected to rise from 135 million in 1995 to 300 million by 2025 (King *et al.* 1998). Insulin resistance is of central importance in the pathogenesis of type 2 diabetes and is also implicated in the development of risk factors for CHD such as abnormal blood lipid patterns. Furthermore, suffering from type 2 diabetes is now recognised to be a risk factor for the development of CHD (Diabetes UK, 2004).

Current trends in health promotion emphasize the importance of reducing dietary fat intake (Department of Health, 1991; FAO/WHO, 2003). However, as dietary fat is reduced, the dietary carbohydrate content of the diet usually increases and the desired reduction in plasma cholesterol concentration is frequently accompanied by an elevation of plasma triacylglycerol (TAG) and reduced HDL-cholesterol concentrations (Mensink & Katan, 1992; Parks & Hellerstein, 2000) that are associated with an increased risk of CHD and type 2-diabetes. Furthermore, results from the previous experimental studies presented in this thesis have shown that increasing carbohydrate intakes (Chapter 3) in postmenopausal women and higher carbohydrate intakes in healthy relatives of individuals with type 2 diabetes and control subjects (Chapter 4) are associated with significantly higher TAG concentrations and lower HDL cholesterol concentrations.

However, it is also evident from the literature (Parks & Hellerstein, 2000; Parks, 2002; Hellerstein, 2002) that not all carbohydrates have the same effects on health, and while some have been shown to have adverse effects on health, especially with regard to lipid levels, it may be that it is the type of carbohydrate that dietary fat is replaced with that is the problem.

Traditionally carbohydrates in foods have been classified as 'simple' or 'complex' depending on how many sugars are in the chain. However, more recently is has been suggested (Jenkins *et al.* 1981) that it may be more useful to describe the effects of carbohydrates on health on the basis of their physiological effects, for example, their ability to raise blood glucose levels, which depend on their constituent sugars (glucose, fructose, and galactose), the physical form of the carbohydrate (particle size and degree of hydration), the nature of the starch (amylose and amylopectin) and other components of the food (dietary fibre, fat). This classification is referred to as the glycaemic index (GI) of a food, which is a method of ranking carbohydrate-containing foods by their blood glucose raising ability (Jenkins *et al.* 1981). A high GI food with an equivalent carbohydrate content as a low GI food induces a larger area under the glucose curve over the postprandial period. Thus, reducing the rate of carbohydrate absorption by lowering the GI of the diet may have several health benefits, such as reduced insulin demand, improved blood glucose control and reduced blood lipid concentrations (Augustin *et al.* 2002).

There is increasing evidence that the GI of carbohydrates is important in the prevention of and control of chronic disease (Brand-Millar, 2002; Frost, 2000; Liu *et al.* 2000). There are reports from a number of the large cohort studies that have

investigated relationships between the dietary GI and glycaemic load (GL; GI x carbohydrate content) and risk of chronic disease. Results from the Nurses' Health study, investigating 75 521 women over a 10-year follow up period, showed that individuals in the highest GL quintile had a significantly increased risk of CHD (OR: 1.98, 95% CI: 1.41, 2.77) (Liu *et al.* 2000). Data from this study following 75 543 women over a 6 year period showed that individuals in the highest GL quintile had a significantly increased risk of developing type 2-diabetes (OR: 1.47, 95% CI: 1.16, 1.86) (Salmeron *et al.* 1997*a*). No significant association was found between dietary GL and type 2-diabetes in the Health Professionals Follow-up study, in which 51, 529 men were followed for 6 years (Salmeron *et al.* 1997*b*). However, individuals who were in the highest quartile for dietary GI had a significantly increased risk (OR: 1.37, 95% CI: 1.02,1.83) of developing the condition (Salmeron *et al.* 1997*b*).

In a cross sectional analysis of the Nurses' Health Study, a strong positive association between GL and fasting plasma TAG levels was observed in postmenopausal women, with a 0.0284 mmol/L increase in TAG per 25-unit increase of GL, along with an inverse relationship with HDL-cholesterol, 0.03 mmol/L reduction per 25-unit increase in GL (Liu *et al.* 2001). In a cross-sectional analysis of the Third National Health and Nutrition Examination Survey and the Survey of British Adults data, significant inverse relationships between GL and HDL-cholesterol were observed (Ford & Liu, 2000; Frost *et al.* 1999). However, in a prospective study of elderly men in the Netherlands these associations were not found (van Dam *et al.* 2000).

A recent review of randomised controlled trials involving individuals with at least one risk factor for CHD by Kelly *et al.* (2004) reported that that low GI diets appear to reduce total cholesterol. In patients with diabetes, intervention studies involving the consumption of low GI diets have generally been found to improve plasma glucose and lipid profiles (Brand-Millar *et al.* 2003). However, whether consuming a diet of low GI is beneficial with regards to the improvement of risk factors for CHD and type 2 diabetes in the healthy non-diabetic population remains controversial. There are very few studies on the effects of low compared with high GI diets in healthy individuals, however, some of these studies have reported beneficial effects of a low versus a high GI diet. In the study of Bouche *et al.* (2002) consumption of a low GI diet for 5 weeks lowered postprandial glucose, insulin and TAG profiles compared with the high GI diet. Sloth *et al.* (2004) reported reduced LDL cholesterol concentrations after a 10-week *ad libitum* low GI diet in overweight healthy subjects. In a study involving six healthy male subjects, Jenkins *et al.* (1987) reported significant reductions in fructosamine (indicator of blood glucose control), 12-h blood glucose profile, 24-h C-peptide concentrations (measure of insulin secretion), and total serum cholesterol concentrations following a low compared with a high GI diets.

Therefore, it appears that there is some evidence from the large cohort studies, cross-sectional studies and from a limited number of intervention studies that consumption of a low GI diet could improve metabolic parameters such as blood glucose, insulin and lipid concentrations and reduce the risk of CHD and type 2 diabetes.

CRP-and IL-6 are acute inflammatory cytokines, and associated with increased risk of CHD (Ridker *et al.* 2002; Pradhan *et al.* 2002) and type 2 diabetes (Hu *et al.* 2004), and whose concentrations in the body appear to be related to glycaemic control. While CRP concentrations are known to be associated with obesity (Visser *et al.* 1999), they have also been independently associated with the $HOMA_{IR}$ index (Wallace *et al.* 2004). In a recently published study conducted in a sample of 1000 middle-aged subjects, CRP levels were positively and independently associated with fasting glucose levels, indicating that hyperglycaemia may also play an important role in the regulation of CRP levels. It has also been reported that CRP concentrations were more strongly associated with post-challenge glycaemia compared with fasting glucose levels (Festa *et al.* 2002). Similarly, IL-6 concentrations have been shown to be associated with insulin sensitivity, independently of BMI (Fernandez-Real *et al.* 2001). Furthermore, Esposito *et al.* (2002) showed that elevating glucose levels to 15 mmol/L for 5 hours while blocking endogenous insulin release significantly increased inflammatory cytokines (IL-6, IL-18, TNF-a) in both normal and impaired glucose tolerant (IGT) subjects.

Thus, there appears to be a relationship between CRP, IL-6 and hyperglycaemia and insulin resistance, and it is conceivable that a diet that leads to better glycaemic control i.e. diets with a lower GI could reduce or be associated with lower levels of CRP and IL-6. I could only find one published report which examined this hypothesis. Liu *et al.* (2002) carried out an analysis on a sub sample of the Women's Health Study Cohort and reported a strong statistically positive association between GL and CRP levels, and the odds ratio for the highest GL quintile compared to the

lowest was 9.43 (95% CI 1.92, 46.23), suggesting that diets with higher GL are associated with higher CRP levels.

Therefore, there appears to be some evidence from the literature that consumption of a low GI diet could reduce the risk of CHD and type 2 diabetes. Thus, it may be that the benefits of reducing fat intake (i.e. cholesterol lowering) could be maintained, and the adverse effects associated with increasing carbohydrate intakes could be avoided if dietary fat was replaced with carbohydrates of low GI. As we have discussed, there are very few intervention studies that have been carried out in healthy individuals and more studies (of both short and long-term) are needed to answer this question. I decided to carry out a short-term study employing healthy subjects whereby dietary carbohydrate intake was increased, and the diets consumed were either of high or low GI.

5.2 Objective

The objective of the study was to examine the influence of high carbohydrate, isocaloric, high and low GI and GL diets for three days on metabolic parameters in the fasted state including plasma lipids, glucose, insulin, NEFA and inflammatory markers in healthy male subjects.

It is much more effective from an economic and a personal cost perspective to prevent chronic diseases such as CHD and type 2 diabetes rather than to treat people who have already developed these conditions. For this reason, it is important to study the effects of different types of diets that may have a role in protecting against the development of these diseases in individuals who are healthy as well as those who already have the conditions or have a high risk. Furthermore, two very recent meta-analyses by Opperman *et al.* (2004) and Kelly *et al.* (2004) have suggested that more studies on the effects of GI on metabolic risk factors among healthy young people free of chronic diseases are needed.

5.3 Methodology

In this study, I was involved in the planning of the study and the development of the intervention diets, I carried out the dietary (Diet 5) analysis of the subjects' compliance to the interventions, and I also carried out the calculations of GI and GL and subsequent analysis of the data.

5.3.1 Study design

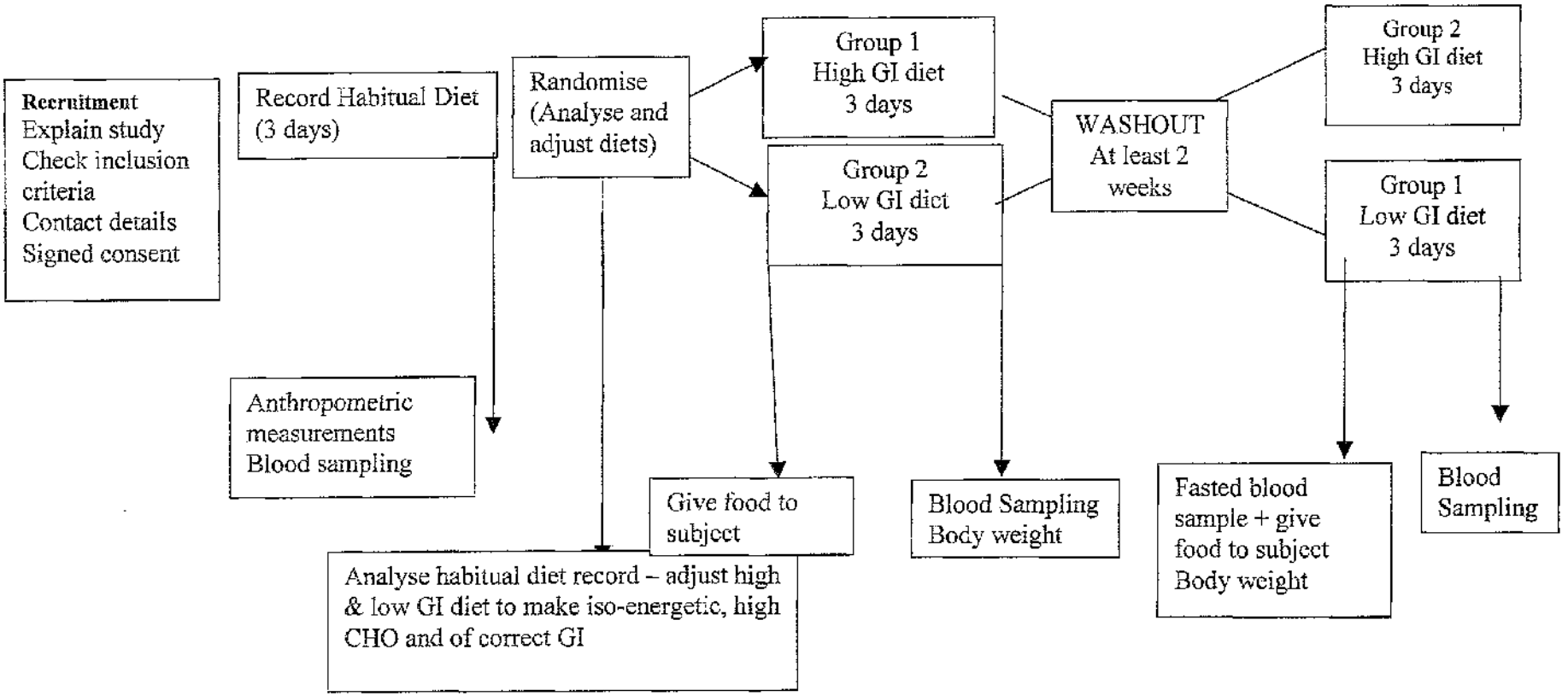
A randomised, crossover design was used in which subjects followed high carbohydrate, high and low GI diets for three days, separated by a washout period of at least two weeks (Figure 5.1). The design of the study was based on that described by Koutsari *et al.* (2000), in which a short-term (i.e. 3-day) high carbohydrate (68% of energy from carbohydrate) diet that was shown to have significant effects on a number of metabolic parameters. Written consent was given by each of the subjects who participated in the study and ethical approval awarded by the University of Glasgow Ethical Committee.

5.3.2 Study outline

Subjects attended five separate visits to the Human Performance Laboratory, located in the Kelvin Building, University of Glasgow. During the first visit, subjects were given written and oral instructions explaining how to complete a 3-day weighed record of their habitual diet. Digital scales and a diary were provided. Subjects also signed the consent form at this visit. Subjects were randomised to start either a high or a low GI diet. At the second visit a fasting blood sample was taken and food for the first dietary intervention was provided, along with individualised version of the menu plans shown in figures 5.2 and 5.3, and careful instructions on how to follow the diet. Anthropometric measurements were also taken during this visit and only body weight was measured at each subsequent visit. The third visit took place, after a 12 hour fast, on the morning after the final day of the first dietary intervention, during which another fasting blood sample was taken. After the washout period the subjects attended their fourth visit to the laboratory where a third fasting blood sample was taken (washout sample) and food, menu plans and instructions for the

second dietary intervention was provided. The final visit took place the morning following the final day of the secondary intervention when a final fasting blood sample was taken. Subjects were instructed not to drink alcohol, change their activity levels or participate in vigorous exercise over the study period, as these are factors known to affect blood lipids.

Figure 5.1 Study Design



5.3.3-Subjects

The subjects who participated in this study were fellow students and friends of the BSc and MSc students who were involved in the study. Fourteen apparently healthy male subjects participated in the study. Subjects were required to fulfil the following inclusion and exclusion criteria:

Inclusion criteria:

- Healthy,
- Normal body weight (i.e. body mass index of between 18.5-25 kg/m^{-2})
- Recreationally active (exercising not more than three times per week)
- Not on weight reducing diet/ weight stable for 1 month prior to testing

Exclusion criteria:

- Taking prescribed medications known to influence lipid metabolism, nutritional supplements and following a special diet (e.g. vegetarian)
- Smoking

5.3.4 Anthropometric measurements

Anthropometric measurements were carried out in the fasted state at the end of the habitual diet-recording period using standard procedures and body weight was measured in the fasted state at each visit. Height (m) (Stadiometer, Holtan Ltd. Crymych) and weight (kg) (SECA scales) were measured and body mass index (BMI) was calculated using the following formula: BMI = weight (kg)/(height (m^2)). Waist, hip and mid upper arm circumferences were measured using standard procedures. Body fat was estimated by skinfold thickness. Three measurements at each of four different sites (biceps, triceps, subscapular and supiliac) in the standing position were taken using callipers (Holtain Ltd. Crymych, UK) and then averaged.

The sum of the four skinfold thickness measurements were used for body fat calculation using the equations of Durnin & Wormersley (1974).

5.3.5 Development and design of the high and low glycaemic index diets

Prior to starting the study, a number of students who participated in the study carried out a 7-day weighed intake of their habitual diet to determine their approximate daily energy intake. The energy intake of the subjects averaged 2,500 kcal per day and the energy content of the standard intervention diets shown figures 5.2 and 5.3 were based on this value. The main high and low GI foods for the intervention diets were then selected using the International table of Glycaemic Index and Glycaemic Load values (Foster-Powell *et al.* 2002). The low GI foods chosen were porridge, wholemeal rye bread, lentil soup, spaghetti (cooked for only 5 minutes) and apple juice. The high GI foods chosen were wholemeal bread, cornflakes, instant mashed potato and lucozade. Initial drafts of the diets were constructed with these high and low GI foods and other foods were added to the diet to make them palatable and to ensure that they would provide adequate energy intake. A substantial amount of time was required to ensure that the high and low GI diets contained similar energy, proportions of energy from carbohydrate, fat and protein, and that the amount of non-starch polysaccharide was as similar as possible. Supermarket research was then carried out to make sure that these foods were readily available and could be purchased within the budget for the study. It was also important the foods contained in the diet did not require too much preparation or cooking.

5.3.6 High and low glycaemic index diets

The intervention diets were designed to be iso-energetic, high in carbohydrate (approximately 70% of energy from carbohydrate), low in fat (approximately 17% energy from fat) and contain equal amounts of dietary fibre, and to differ only in GI and GL. The nutritional composition of the diets is shown in table 1. Menus of the standard intervention diets are provided in Figure 5.2 and 5.3, and the major high and low glycemic foods highlighted in bold. As already mentioned, subjects completed a 3-day diet record of their habitual diet. These diaries were analysed using Diet 5. A new individually planned diet was created based on the standard diets, but modified so that it would be iso-energetic with the habitual diet of the subjects. We also could have predicted the subjects' energy requirements using the Schofield *et al.* (1985) equations to estimate BMR and by adjusting for a moderate level of physical activity. Using of this method would have avoided the problems associated with under-reporting. However, we were also able to use the 3-day diet records to examine the quality of the diet and the GI and GL.

5.3.7 Dietary analysis, calculation of glycaemic index and glycaemic load

Dietary analysis of the habitual and intervention diets was carried out using a computerised version (Diet 5^{TM}, Robert Gordon University, Aberdeen) of the food composition tables (Holland *et al.* 1991). GI values were taken from the International table of GI and GL values (Foster-Powell *et al.* 2002). GL of individual foods were calculated by multiplying the amount of carbohydrate, in grams, in each food (obtained from Diet 5^{TM}, Robert Gordon University, Aberdeen) with their respective GI value and dividing by 100. The GL for each diet was obtained by finding the sum of each individual GL value. The GI of each diet was calculated by dividing the GL

of the diet by the total amount of carbohydrate in the diet (obtained from Diet 5^{TM}) then multiplying by 100.

Table 5.1 Nutritional Composition of the high and low glycaemic index diets

	High GI diet	Low GI diet
Energy (Kcal)	2524	2531
Percentage energy from		
CHO	69.9	70.7
Fat	17.1	17.5
Protein	13.0	11.8
NSP (g)	25.2	24.5
GI	77	35
GL	358	155

Figure 5.2. High Glycaemic Index Diet

Breakfast
Wholemeal bread (2 slices) with **jam** (40g) and low fat spread (14g)
Mug tea (260g) with semi-skimmed milk (40g)
Corn Flakes (55g) with semi skimmed milk (100g)

Morning Snack
Apple (150g)

Lunch
Ham sandwich (made with 4 slices **wholemeal bread** (140g) with low fat spread (28g) and lean ham (48g)
Glass of **lucozade** (330g)

Afternoon snack
Glass of **lucozade** (330g)

Evening meal
Glass of **lucozade** (330g)
Low fat chicken casserole (200g)
Instant potato cooked (240g) with 10g of low fat spread
Peas boiled (65g)

Evening snack
Wholemeal bread (2 slices) with jam (40g) and low fat spread (14g)
Mug tea (260g) with semi-skimmed milk (40g)

Figure 5.3. Low Glycaemic Index Diet

Breakfast
Glass **apple juice** (330g), Mug tea (260g) with semi-skimmed milk (40g)
Bowl **porridge** (made with semi-skimmed milk) (300g)
Wholemeal rye bread (2 slices) + low-fat spread (14g)

Morning Snack
Banana (green/under-ripe) (120g), Apple (150g)

Lunch
Ham sandwich (made with rye bread (50g) with low fat spread (14g) and lean ham (48g)
Can **lentil soup** (400g), **apple juice** (330g)
Apple (150g)

Afternoon snack
Banana (green/under-ripe) (120g), Apple (150g)

Evening meal
Spaghetti (boiled for only 5 minutes) (400g cooked weight)
Pasta sauce (355g), **apple juice** (330g)
Low fat fruit yogurt (200g)

Evening snack
Wholemeal rye bread (50g) with low fat spread (14g) + 2 slices processed cheese (40g)
Glass water

5.3.8 Compliance

The diets were designed to ensure maximum compliance by using foods that were easy to prepare and involved little cooking. All foodstuffs were provided to the subjects and in many cases delivered to their homes. Subjects were provided with individually tailored versions of the menus plans shown in figures 5.2 and 5.3 along with clear written and oral instructions, and subjects were able to make at any time by telephone if they had any queries. Subjects were encouraged to follow the individually tailored menu plans as closely as possible and to note changes, if any, which were returned to the investigators during the laboratory visit following the dietary intervention and analysed using Diet 5^{TM} (Robert Gordon University,

Aberdeen) to assess reported compliance to the prescribed diets. GI and GL of the actual diets were calculated as already described. I carried out the analysis of compliance to the diets myself.

5.3.9 Blood sampling

Blood sampling was carried out by my supervisor using a cannula inserted into a forearm vein. A cannula was used as subjects took part in an exercise trial involving additional blood sampling after the fasted blood sample was obtained. Fasted blood samples were taken into EDTA (2 x 7ml) and lithium heparin (1 x 6ml) vacutainers and were centrifuged (Mistral 3000i, Sanyo Gallenkamp plc, Leicester UK) at 3,000 rpm for 10 min at 4 ^{0}C. After separation, plasma was alliquoted into pre-labelled ependorf tubes that were then stored at -70 ^{0}C for analysis later on. EDTA plasma was used for the analysis of plasma lipids, glucose, NEFA, CRP and IL-6, while lithium heparin plasma was used for the analysis of insulin.

5.3.10 Laboratory analysis

The majority of the laboratory analysis for this study was carried out at the Department of Pathological Biochemistry (lipids, glucose, NEFA, CRP) or the Department of Biochemistry (insulin) at the Glasgow Royal Infirmary. For this reason only a brief indication of the methods are provided here and full details of each of the methods are given in Chapter 2. Interleukin-6 measurements were carried out at the Department of Human Nutrition and the method is described below. Plasma samples collected into EDTA were used for determinations of TAG, total cholesterol, HDL cholesterol, non-esterified fatty acid (NEFA) and glucose by standard enzymatic colorimetric procedures (Roche Diagnostic Corporation, Lewes,

UK). Low-density lipoprotein (LDL) cholesterol concentrations were calculated using the Friedelwald equation (Friedelwald *et al.* 1972). Insulin concentrations were determined from lithium heparin plasma using an automated analyser technique (Abbot IMX) and dry slice technology. Analysis of C-reactive protein was carried out in EDTA plasma samples using an in house ELISA (Highton & Hessian, 1984). Finally, from the fasting insulin and fasting glucose values, the HOMA index was calculated (fasting insulin x fasting glucose/22.5) and used as a measure of insulin resistance (Matthews *et al.* 1985).

Interleukin-6 (IL-6) determination was performed using a quantitative sandwich enzyme linked immunosorbent assay (ELISA) kit (R&D Systems, Europe Ltd). The Quantikine High Sensitivity kit was chosen since all our subjects were healthy individuals and no extreme values were expected. The analysis was performed at the Department of Human Nutrition laboratory in Yorkhil hospital by an MSc student (Dimitrios Kessaris), one of my supervisors (Dr Siobhan Higgins) and with the help of an experienced technician (Alexander Fletcher). The full method is described in the methodology chapter (Chapter 2).

5.3.11 Statistical analysis

I carried out a power calculation before starting the study to find out how many subjects would be needed to see a statistically significant difference in fasting TAG levels. The information for the power calculation was collected in my first study and I used baseline and week one of the intervention TAG concentrations to calculate the mean difference and the standard deviation of the difference (mean difference = –0.046; standard deviation of the difference = 0.0529; Power = 80%). The results of

this power calculation showed that at least 8 subjects would be needed in the present study.

As mentioned above, a total of fourteen subjects completed the trial, however not all of these subjects were included in the statistical analysis and the reasons for that are given here. The results for one of the subjects were excluded from analysis as this subject was found to have dyslipidemia according to the cut off values (TAG > 2.26 mmol/L, total cholesterol > 6.22 mmol/L and LDL-cholesterol > 4.14) defined by the National Cholesterol Education Program (NCEP) (2001). Thus, the number of subjects that I have for the dietary, lipid, glucose, insulin and NEFA results for is thirteen. There were two other subjects for which results for CRP and three subjects for which IL-6 results were not analysed and therefore the subject number is 11 for CRP and 10 for IL-6.

Data were expressed as mean ± standard deviation (SD) for normally distributed variables and median and range for non-parametric variables. Data was checked for normality by inspecting the histograms in SPSS. All variables apart from CRP and IL-6 data were normally distributed. Where data were normally distributed, paired *t*-test was used to compare mean values before and after dietary intervention for significant differences. Where data was not normally distributed, the comparison was made using Wilcoxon Signed Ranks test. Statistical analysis was performed using SPSS (version 11.0) and P values less than 0.05 were considered to be statistically significant.

5.4 Results

5.4.1 Subject characteristics

The mean body mass index (BMI) for the group was within the healthy range (20-25 $kg.m^{-2}$), however, three of the thirteen subjects were classified as overweight with a BMI over 25 $kg.m^{-2}$. However, these subjects were not excluded from the study as their waist circumference (<102 cm ; Janssen *et al.* 2004) and percentage body fat (<25%) were within the acceptable ranges for good health (American Council on Exercise, 2004) (Table 5.2). Body weight remained constant over each study period.

Table 5.2. Subject characteristics at baseline (n=13)

Characteristics	Mean	SD	Min	Max
Age (years)	24.2	4.5	19.0	33.0
BMI ($kg.m^{-2}$)	23.9	2.0	20.2	26.9
Waist circumference (cm)	79.8	5.0	71.4	87.5
Waist to hip ratio	0.84	0.05	0.78	0.94
Body fat (%)	15.0	2.7	10.7	19.2
Mid upper arm circumference (cm)	31.0	3.4	23.0	35.0

5.4.2 Daily energy and macronutrient intakes (absolute amounts)

The low and high glycaemic index (GI) intervention diets consumed were significantly higher in carbohydrates, sugar, non-milk extrinsic sugar, starch, non-starch polysaccharides and protein compared with the subjects' habitual diet. The intervention diets consumed were significantly lower total fat, polyunsaturated fat and alcohol compared to the subjects' habitual diet. The low GI diet was significantly lower in saturated and monounsaturated fat compared to the subjects habitual diet. As expected, the GI of the low GI diet was significantly lower that the habitual diet and the GI of the high GI intervention diet was significantly higher

compared to the subjects habitual diet. The glycemic load (GL) of the high GI diet was significantly higher that the subjects habitual diet (Table 5.3).

While the high and low GI intervention diets were designed to be similar nutritionally with exception of GI and GL, the low GI diet consumed was found to be significantly higher in the intake of sugars (P=0.001) and polyunsaturated fat (P=0.006) compared to the high GI diet (Table 5.3).

5.4.3 Percentage of energy from the main energy producing macronutrients in habitual, low and high GI diets

The percentage of energy derived from total fat and each of the different types of fat and protein in both intervention diets consumed by the subjects was significantly lower compared with subjects' habitual diet. The proportion of energy from carbohydrates, sugars, non-milk extrinsic sugar, and starch was significantly higher in both low and high GI diets compared with habitual diet.

There were no differences in percentage of energy from carbohydrates, non-milk extrinsic sugar, starch, and non-starch polysaccharides between the intervention diets. However, the mean percentage of energy from sugar was significantly greater in the low compared with high GI diet (P=0.001). There was no significant difference in percentage of energy from total fat, saturated and monounsaturated fat between low and high GI diets while the percentage of energy from polyunsaturated fat was significantly lower in the high GI diet compared with the low GI diet (P=0.011). No significant difference was found in other energy-producing macronutrients between the two intervention diets (Table 5.4).

Table 5.3. Daily energy and macronutrient intakes, glycaemic index and glycaemic load of subjects' habitual and intervention diets (n=13)

	Habitual		Low Glycaemic Index diet		*P**	High Glycaemic Index diet		*P***
	Mean	SD	Mean	SD		Mean	SD	
Energy (kcal)	2468	271	2564	300	0.131	2547	306	0.286
Energy (Kj)	10716	1256	10646	1277	0.131	10318	1134	0.286
Total fat (g)	92.6	19.6	49.3	6.8	**0.003**	46.6	5.6	**0.003**
Saturated fat	33.8	10.8	14.6	3.1	**0.003**	13.7	1.4	0.151
Monounsaturated fat	31.8	9.8	13.7	3.1	**0.003**	13.8	2.1	0.753
Polyunsaturated fat	17.0	3.7	11.9	1.8	**0.001**	10.8	1.6	**0.008**
Carbohydrate (g)	289.7	46.6	450.5	54.8	**0.003**	448.9	60.1	**0.003**
Sugar	119.5	49.7	260.4	44.0	**0.003**	177.4	39.2	**0.016**
Non-milk extrinsic sugar	79.6	49.2	141.0	15.6	**0.016**	140.6	24.4	**0.016**
Starch	140.7	40.5	190.2	21.1	**0.010**	182.6	23.4	**0.006**
Non-starch polysaccharide	17.3	6.8	24.3	3.6	**0.026**	24.5	4.6	**0.026**
Protein	110.2	22.9	79.4	13.2	**0.004**	82.8	7.7	**0.003**
Alcohol	5.0	7.7	0	0	**0.043**	0	0	**0.043**
Glycaemic index	55.9	4.4	36.2	1.1	**0.003**	77.0 [a]	2.7	**0.003**
Glycaemic load	159.1	30.8	162.1	18.6	0.657	332.3 [b]	83.8	**0.004**

*** Wilcoxon Signed Ranks test for comparison between habitual and low GI diet**

**** Wilcoxon Signed Ranks test for comparison between habitual and high GI diet**

[a] ***P*=0.001**

[b] ***P*=0.002**

Table 5.4. The percentage of energy intake from macronutrients of subjects' habitual and intervention diets (n=13)

	Habitual		Low Glycaemic Index diet		*P**	High Glycaemic Index diet		*P***
	Mean	SD	Mean	SD		Mean	SD	
% Energy from:								
Total fat	33.7	5.1	17.3	1.4	**0.003**	16.5	1.4	**0.003**
Saturated fat	12.2	3.2	5.1	1.0	**0.003**	4.9	0.3	**0.003**
Monounsaturated fat	11.4	2.7	4.8	0.9	**0.003**	4.9	0.6	**0.003**
Polyunsaturated fat	7.1	3.0	4.2	0.5	**0.005**	3.8[a]	0.4	**0.004**
Carbohydrate	46.9	5.4	70.3	2.1	**0.003**	70.4	1.8	**0.003**
Sugars	19.3	7.4	40.5	3.7	**0.003**	27.6[b]	3.7	**0.026**
Non-milk extrinsic sugar	12.9	7.5	22.2	2.9	**0.010**	21.9	1.8	**0.010**
Starch	22.7	5.8	29.8	2.3	**0.013**	29.7	3.6	**0.006**
Protein	17.9	3.4	12.4	1.2	**0.003**	13.1	0.7	**0.003**
Alcohol	1.5	2.3	0	0	**0.043**	0	0	**0.043**

*** Wilcoxon Signed Ranks test for comparison between habitual and low GI diet**
**** Wilcoxon Signed Ranks test for comparison between habitual and high GI diet**
[a] **P=0.011**
[b] **P=0.001**

5.4.4 Fasting plasma lipid concentrations

There were reductions in total cholesterol, HDL cholesterol level and LDL cholesterol following low GI diet (P=0.029, P=0.009 and P=0.058, respectively) compared with baseline values. After the high GI diet, HDL cholesterol concentration was also significantly lower than baseline. However, the ratio of total cholesterol to HDL cholesterol was significantly higher (P=0.009) after the high GI diet compared with baseline. TAG levels were significantly increased after both the high and low GI diets compared to baseline. TAG concentrations were significantly higher after the low GI diet compared with after the high GI diet (P=0.004). There were no significant differences in any of the other plasma lipids after the intervention diets (Table 5.5).

5.4.5 Fasting NEFA, glucose, insulin, $HOMA_{IR}$

There were no significant changes in fasting NEFA, glucose, insulin concentrations or $HOMA_{IR}$ score after the low or high GI diets compared with baseline values. Furthermore, there were no significant differences in any of these parameters between the low and high GI diet interventions (Table 5.6).

5.4.6 Fasting C-reactive protein (CRP) and interleukin-6 (IL-6) concentrations

As the data for IL-6 and CRP were not normally distributed, the median and range for the data is presented (Table 5.7). The medians for CRP were higher after both the low and high GI diets compared with baseline values, however the changes were not statistically significantly. However, CRP concentrations after the low GI diet were almost significantly higher (P=0.075) compared with baseline concentrations. When the individual CRP concentrations were examined, CRP concentrations were found

to be higher after either high (6/11) or low (7/11) GI diets in 9 of the 11 subjects (Figure 5.4).

The medians for IL-6 concentrations were also higher after both the low and high GI diets compared with baseline values, however the differences were not statistically significant. When the IL-6 concentrations were examined for each individual (Figure 5.5), it was found that IL-6 concentrations were higher after either low (5/10) and high (6/10) in 7 of the 10 subjects for which IL-6 results were available.

Table 5.5 Fasting plasma lipid concentrations at baseline, low and high glycaemic index diets (n=13)

	Baseline		Low Glycaemic index diet		*P**	High Glycaemic index diet		*P***
	Mean	SD	Mean	SD		Mean	SD	
Triacylglycerol (mmol.l^{-1})	0.83	0.27	1.27	0.53	**0.001**	0.96[a]	0.40	**0.037**
Total cholesterol (mmol.l^{-1})	4.16	0.67	3.90	0.70	**0.029**	3.98	0.67	0.197
HDL cholesterol (mmol.l^{-1})	1.40	0.26	1.26	0.26	**0.009**	1.28	0.32	**<0.001**
LDL cholesterol (mmol.l^{-1})	2.59	0.72	2.38	0.69	0.058	2.52	0.70	0.902
TC: HDL cholesterol ratio	3.07	0.72	3.22	0.93	0.128	3.27	0.92	**0.009**

*** *P* for comparison between baseline and low glycaemic index diet (Paired *t*-test)**
**** *P* for comparison between baseline and high glycaemic index diet (Paired *t*-test)**
[a] Significantly different from low GI diet ($P = 0.004$)

Table 5.6 Fasting non-esterified fatty acids (NEFA), glucose and insulin concentrations and $HOMA_{IR}$ at baseline, low and high glycaemic index diets (n=13)

	Baseline		Low Glycaemic Index diet		*P**	High Glycaemic Index diet		*P***
	Mean	SD	Mean	SD		Mean	SD	
NEFA ($mmol.l^{-1}$)	0.39	0.17	0.48	0.37	0.461	0.40	0.23	0.924
Glucose ($mmol.l^{-1}$)	5.02	0.54	5.11	0.39	0.588	4.92	0.30	0.473
Insulin ($\mu U.ml^{-1}$)	6.76	1.87	7.12	3.05	0.469	7.15	3.00	0.554
$HOMA_{IR}$	1.52	0.49	1.64	0.77	0.389	1.58	0.73	0.714

*** *P* for comparison between baseline and low glycaemic index diet (Paired *t*-test)**

**** *P* for comparison between baseline and high glycaemic index diet (Paired *t*-test)**

Table 5.7 C-reactive protein and Interleukin-6 concentrations at baseline, low and high glycaemic index diets (n=11)

	Baseline		Low Glycaemic Index diet		*P**	High Glycaemic Index diet		*P***
	Median	Range	Median	Range		Median	Range	
C-reactive protein ($mg.L^{-1}$)	0.25	(0.00,0.82)	0.46	(0.00,2.47)	0.075	0.47	(0.00,2.73)	0.314
Interleukin-6 (pg/ml)	0.68	(0.46,1.35)	0.74	(0.51,2.27)	0.859	0.77	(0.45,1.88)	0.440

*** *P* for comparison between baseline and after low glycaemic index diet (Wilcoxon Signed Ranks test)**

**** *P* for comparison between baseline and after high glycaemic index diet (Wilcoxon Signed Ranks test)**

Figure 5.4. Individual C-reactive protein concentrations at baseline, after low and high GI diets

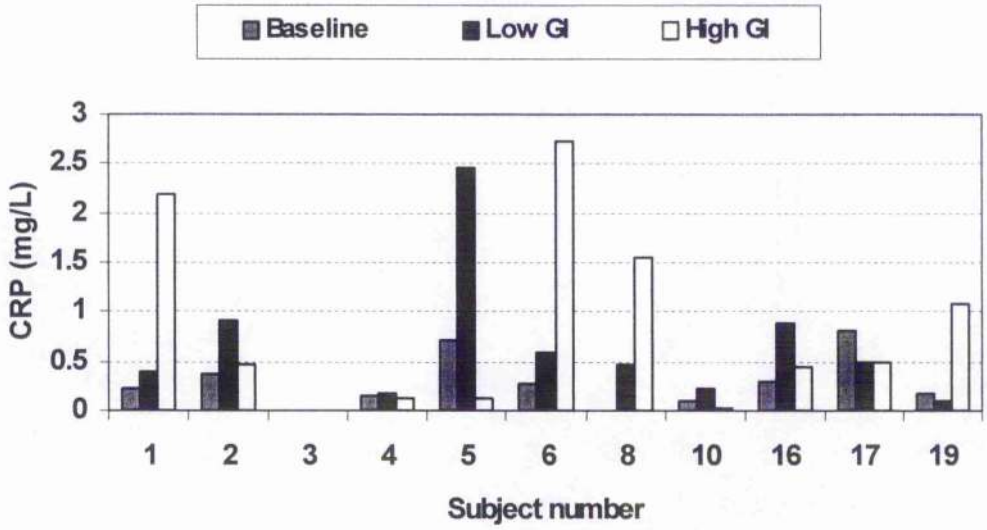
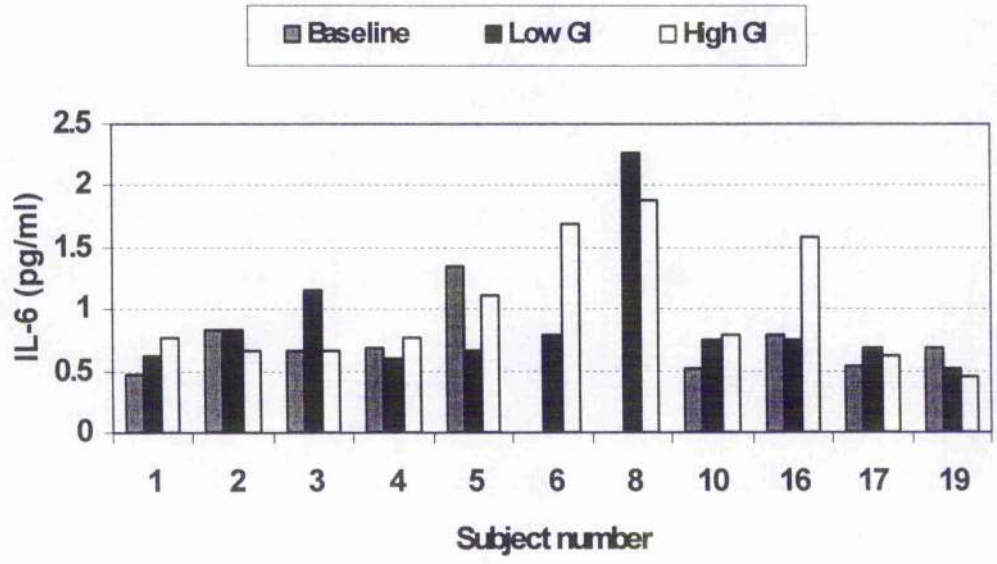


Figure 5.5. Individual interleukin-6 concentrations at baseline, after low and high GI diets.



5.5 - Discussion

The aim of this study was to investigate the short-term effects of two high-carbohydrate, low-fat high and low glycaemic index (GI) diets on a number of fasting metabolic parameters using a randomised crossover design in healthy male subjects.

Reported compliance to the intervention diets showed that as planned, the low and high GI diets were of equal energy content with the habitual diets, and the intervention diets were significantly higher in carbohydrate (increased from 46.9% to ≈70% of energy) and lower in fat (reduced from 33.7% to ≈17% of energy) compared with the habitual diets. Furthermore, the GI values of the low (36.2 ± 1.1) and high (70.0 ± 2.7) GI diets consumed were significantly different from that of the habitual diet (55.9 ± 4.4) and were significantly different from each other. The glycaemic load (GL) of the high GI diets (332.3 ± 83.8) consumed were also significantly higher compared with those of the subjects' habitual (159.1 ± 30.8) and low GI diets (162.1 ± 18.6). Thus, subject compliance to the prescribed diets in this study appears to have been very good.

The main findings of the present study were that both high-carbohydrate, low-fat intervention diets significantly increased fasting plasma TAG and significantly reduced HDL cholesterol concentrations, and the low GI diet also reduced total and LDL cholesterol (almost significant; $P = 0.058$) concentrations. Furthermore, the increase in fasting plasma TAG was greater after the low GI diet compared with the high GI diet. There were no significant changes in fasting plasma NEFA, glucose or insulin concentrations, or in the $HOMA_{IR}$ score following either of the intervention

diets. Although no statistically significant changes in either IL-6 or CRP were observed following either the high or low GI diets, there does appear to be a trend for both of these high carbohydrate diets to have increased these inflammatory markers.

The increase in fasting plasma TAG and the reduction in HDL cholesterol concentrations observed after both of these high-carbohydrate, low-fat intervention diets are consistent with many previous reports (Mensink & Katan, 1992; Parks *et al.* 1999). This finding is also in agreement with a study of similar design in which switching to a high carbohydrate diet (68% of total energy intake) for three days resulted in an increase in fasting TAG and a reduction in HDL cholesterol concentration (Koutsari *et al.* 2000).

The mechanism for the increase in TAG after consumption of a high carbohydrate diets is debated but is most likely due to an increase in hepatic secretion and synthesis of VLDL-TAG (Blades & Garg, 1995; Mittendorfer & Sidossis, 2001). The two mostly likely sources of the fatty acids used for TAG synthesis are believed to be firstly, fatty acids derived from the plasma non-esterified fatty acid pool, which in the fasted state, mainly come from adipose tissue; and, secondly, fatty acids synthesised from glucose in the liver or *de novo* lipogenesis (Parks *et al.* 2002). It has also been suggested that a reduction in TAG clearance may occur following a high-carbohydrate diet, and there are two mechanisms by which TAG clearance could be effected (Parks *et al.* 1999). The first is via lipoprotein lipase, an enzyme that hydrolyses core TAG off circulating chylomicrons and VLDL. One hypothesis is that increases in insulin after high carbohydrate diet decreases lipoprotein lipase activity in muscle, leading to decreased TAG clearance. The second mechanism by

which TAG clearance from plasma is believed to occur is via a receptor mediated process (which could be down-regulated by a high-carbohydrate diet) in which TAG-rich lipoproteins are taken up by the liver (Parks *et al.* 2002). Which of these mechanisms is most important when high carbohydrate diets cause an elevation in TAG is not currently known.

Elevated TAG concentrations are associated with lowered HDL cholesterol concentrations as was found following both intervention diets in the present study. Increased TAG concentrations due to an increased carbohydrate intake have been shown to lead to adverse changes in HDL and LDL cholesterol via excessive transfer of TAG catalysed by the action of cholesterol ester transfer protein (CETP). The accumulation of excessive amounts of TAG on HDL and LDL leads to the removal of TAG from these lipoproteins via hepatic lipase resulting in the formation of small, dense lipoprotein particles (Gibney *et al.* 2003). In the case of HDL, smaller, more dense particles are more quickly broken down by the liver leading to reductions in circulating levels as we observed in the present study. A second explanation for the lower HDL cholesterol concentrations observed might be the reduced need for cholesterol to be removed out of circulation due to the lower dietary saturated fat intake of the high-carbohydrate, low-fat intervention diets (Velez-Carrasco *et al.* 1999). On the other had, small, dense LDL remains in circulation for longer as it is not well recognised by the LDL receptor. Due to its small size and the fact that it remains in circulation for longer periods than normal, it is better able to penetrate the endothelium and contribute to the development of atherosclerosis (Gibney *et al.* 2003).

The results this study appear to suggest that a high carbohydrate diet of low GI has more harmful effects on fasting plasma TAG, short-term, than a high carbohydrate diet of high GI, as TAG concentrations were significantly higher after the low GI diet compared with the high GI diet (P = 0.004). This adverse effect on fasting TAG after the low GI diet was unexpected. Numerous intervention studies have examined the effects of dietary GI on TAG in individuals with diabetes under macronutrient-controlled condition, and the majority reported that plasma TAG was reduced by a low GI diet (Ludwig, 2002). However, a recent Cochrane review (Kelly *et al.* 2004) reported no evidence of an effect of a low GI diet on plasma TAG in individuals with at least one risk factor for or established CHD. I could find no other study on healthy subjects of similarly short duration comparing low and high GI diets on plasma TAG, and studies in which the intervention period was of longer duration (ie. two weeks or more) have reported either no significant differences (Jenkins *et al.* 1987; although TAG was reduced in five of the six subjects studied) or changes (Sloth *et al.* 2004), or significantly reduced postprandial TAG (Bouche *et al.* 2002).

The low GI diet was higher in sugar compared with the high GI diet, and this may be the reason for the adverse effect on TAG, as sugars have been reported to have a more adverse effect on TAG than other types of carbohydrate (Parks and Hellerstein, 2000). The amount of sugar calculated (using Diet 5) to be in the low GI diet was significantly higher (260.4g ± 44.0g; $P = 0.001$) compared with the high GI diet (177.4g ± 39.2g) (Table 5.3). There was approximately an 83g calculated difference in the amount of sugar between the diets. This seems to be related to the amount of intrinsic food sugars rather than the non-milk extrinsic sugars (NMES) supplied by foods such as apple juice, lucozade, pasta sauce or yoghurt. In fact there was no

difference calculated in the NMES contents of the intervention diets (140.6 ± 24.4 vs. 141.0 ± 15.6; NS) (Table 5.3). The low GI diet in this study contained two bananas (weighing 240g; 20.9g sugar per 100g banana = 50.2g sugar) and two apples (weighing 300g; 11.8g sugar per 100g apple = 35.4g) more than in the high GI diet, which seems to have supplied this additional sugar. The food tables show that the carbohydrate contained in bananas is almost all sugars (Holland *et al.* 1991), and in particular sucrose, galactose and fructose (Forster *et al.* 2002). Unfortunately I did not carry out a chemical analysis of the intervention diets. However, Englyst & Cummings (1992) studied the digestion and absorption of the carbohydrate in bananas by feeding ileostomy subjects bananas of different degrees of ripeness. The authors reported a ten-fold difference in non digestible starch in very ripe and not so ripe bananas with 37% of the dry weight in the least ripe being non digestible starch to only 3% of the dry weight in the bananas that were most ripe. Thus, with progressive ripeness there is a decrease in starch and an increase in the sugar content of bananas (Ercan *et al.* 1993). As the bananas used in the study were green (therefore under-ripe) they probably contained much more non-digestible starch than sugar. Thus the difference in the sugar contents of the diets may have been over-estimated, and the low GI diet may only have contained about 35g of sugar (from the apples) more than the high GI diet. Thus, it may have been this additional sugar supplied by the two extra apples that caused the more adverse effect on TAG of the low GI diet.

In the present study, there was a reduction in total cholesterol ($P = 0.029$) and LDL cholesterol (almost significant, $P = 0.058$) after the low GI diet but not after the high GI diet. This finding is in agreement with most previous studies which have show

that a low GI diet reduces either total or LDL cholesterol or both parameters. Ludwig (2002) reviewed twelve studies involving diabetic and hyperlipidemic patients, and reported that in all but one of theses studies, a low GI diet was associated with lower LDL cholesterol concentrations. In the recent review by Kelly *et al.* (2004), a meta-analysis of pooled data from thirteen studies showed a mean reduction in total cholesterol of 0.17mmol/L, P = 0.03 (95% confidence interval –0.32 to –0.02) but no evidence of an effect of low GI diets on LDL cholesterol. In the studies that have been carried out on healthy individuals, Jenkins *et al.* (1987) reported a reduction in total cholesterol of 15 ± 3% ($P < 0.01$) after the low GI diet. Sloth *et al.* (2004) reported a 10% decrease ($P < 0.05$) in LDL cholesterol and a tendency for a larger reduction in total cholesterol ($P = 0.06$) with consumption of the low GI as compared with the high GI diet. Bouche *et al.* (2002) reported a tendency for a reduction in total cholesterol ($P = 0.065$) as well as apo B ($P = 0.076$) after the low GI intervention of their study. A reduction of fat intake, especially saturated fat intake, as occurred with both intervention diets in this study, is known to reduce cholesterol levels. A reduction in saturated fat is believed reduce cholesterol in the circulation by up-regulating the LDL receptors in the liver to increase the uptake of cholesterol out of circulation and thus reducing total plasma levels and that carried by the lipoprotein fractions (Conor & Conor, 1997; Gibney *et al.* 2003). However, this could explain why both intervention diets would have reduced cholesterol not why the low GI diet only had these effects.

Our results appear to show a trend towards increased levels of inflammatory markers after both high carbohydrate intervention diets with no clear difference between the high and low GI interventions. CRP concentrations were non-significantly increased

after-both the low (P=0.075) and high GI diets compared with baseline values, and individual CRP concentrations higher in 9 of the 11 subjects after the dietary interventions. Similarly, median IL-6 concentrations were non-significantly increased after both diets compared with baseline values, and IL-6 concentrations were higher in 7 of the 10 subjects.

However, there were a number of factors associated with the design of this study which may not have made it the most suitable for investigating the effects of these diets on inflammatory markers. This study was initially designed primarily to examine the effect of the diets on plasma lipids and the power calculation was carried out using data for TAG concentrations. However, a power calculation was carried out retrospectively using the IL-6 data, and this showed that 25 subjects would have been necessary to identify significant changes in IL-6. Thus, it is very likely that the present study did not have the statistical power to see a difference in inflammatory markers between these two diets. Also, the length of dietary interventions was based on the time needed to see a change in plasma lipids, and as there is no other similar study in the literature, it is not known the length of dietary intervention that would be optimal, if any, to observe a change in inflammatory markers with high and low GI diets.

There were no changes in fasting NEFA, glucose or insulin concentrations, or in $HOMA_{IR}$ observed in the present study. Fasting concentrations of these metabolites are well regulated, and this may be the reason that we were not able to observe any changes. In addition, the fact that healthy individuals were employed as subjects made it more difficult to see changes in these metabolites (Parks & Hellerstein,

2000) However, it could be speculated that the changes in fasting lipid concentrations and the trend towards increases in the inflammatory markers observed were due to changes in the day time profiles of these metabolites during the three-day dietary intervention. For example, increasing the carbohydrate contents of the diets to approximately 70% energy may have resulted in higher day-time profiles of glucose and insulin, especially on the high GI diet. Unfortunately, we were not able to measure the day-time profiles of these metabolites at part of the present study, however, postprandial changes that occur during high and low GI diets are clearly shown in a number of other studies (Jenkins *et al.* 1987; Kiens & Richer, 1996). Jenkins *et al.* (1987) reported that while fasting glucose concentrations were not different after twelve days on a high GI diet compared with a low GI diet, 12-hour blood glucose profiles were found to be significantly higher. Similarly, in the study of Kiens & Richer (1996), while morning fasting insulin concentrations were not different after high and low GI diets, serum insulin concentrations were found to be significantly lower after the lunch-time meal after three days on a low GI diet compared with the high GI diet.

There were a number of limitations associated with this study. Ideally, I would have like to have measured lipids not only in the fasted state but also collected postprandial blood samples. It is generally agreed ((Zilversmit, 1979; Chen *et al.* 1995; Jeppessen *et al.* 1995; Frayn *et al.* 1997; Frayn,1998a; 1998c; Koutsari *et al.* 2000; Lemieux *et al.* 2000; Yu and Cooper 2001; Daly 2003; Wolever and Mehling, 2003a; Wolever *et al.* 2004; Parks *et al.* 2002) that disease risk is better predicted by a postprandial test, especially in the case of TAG, as most individual are in the postprandial state for 16-18 h/d and only in the fasted state for about 6 h in the

middle of the night. Unfortunately, collection of postprandial blood samples was not possible in this study.

The calculation of GI and the GL of the diets was performed using published glycaemic index tables (Foster-Powell *et al.* 2002) rather than actually measuring the GI of the foods or meals or diets themselves. In addition, while the tables provided data for all the foods used in the two intervention trials some of the foods of the habitual diets of the subjects were missing from the tables. As mentioned above, in that case GI values of similar foods were used. These factors may have led to incorrect estimations of the GI and the GL of the diets and for this reason the difference between the two intervention diets and the habitual diet may not have been as great as estimated.

Another limitation of our study is that we used a three day weighed diet record to assess subjects' habitual energy intake. Ideally we would have used the doubly labelled water method to assess habitual energy expenditure, which is recognised as the more objective measure of habitual energy intake. However, this technique is expensive and was beyond the scope of this study. However, we could have asked subjects to record their habitual diets for at least seven days which would have been more reliable than three days..

In conclusion, the results of this study showed a beneficial effect of a low versus a high GI diet on total and LDL cholesterol concentration. However, as regards effects on TAG and HDL cholesterol, no benefits of a low GI were found, but this was probably due to the high carbohydrate content of the diets. Surprisingly, the low GI

diet appeared to have a more adverse effect on TAG which may be explained by the fact that the low GI diet was higher in sugar. Overall, the results suggest that in a high carbohydrate diet, the total carbohydrate and sugar content of the diet may have a more important influence on lipids and other metabolic parameters than the glycaemic index of the diet. That is not to say that glycaemic index of the diet is not important, and there are several epidemiological studies which support a beneficial effect low GI diets (Salmeron *et al.* 1997a; Salmeron *et al.* 1997b; Frost *et al.* 1999; Liu *et al.* 2000; Ford & Liu, 2000; Liu *et al.* 2001). Furthermore, diets of low GI tend to be associated with other healthy dietary attributes, for example, they tend to contain more wholegrain, more non-starch polysaccharide, antioxidants and other micronutrients, and tend to be lower in fat. This was an experimental, mechanistic type study and future studies should look at the effects of glycaemic index diets in diets with a more realistic carbohydrate content (50% energy) which would have more relevance to real life situations and to public health.

Chapter 6

General discussion and conclusion

6.1 Introduction

Coronary heart disease (CHD) is leading cause of death and ill health worldwide (FAO/WHO, 2004), and is the most common cause of mortality in the United Kingdom (British Heart Foundation, 2004), and it is a particularly serious problem in Scotland (The Scottish Office, 2000). Type 2 diabetes is also a major public health concern with the rates of this condition increasing rapidly worldwide (King *et al.* 1998), and worryingly the disease has also started to appear in children (Aboderin, 2001). Also, individuals who develop type 2 diabetes have a high risk of developing CHD (Niskanen *et al.* 1998)

Insulin resistance, which is the term given to the situation in which the actions of insulin are blunted in the presence of normal or increased insulin secretion (Gibney *et al.* 2005), is a central feature in the development of type 2 diabetes and is now recognised to play a role in many of the risk factors for CHD such as abnormal lipid levels or dyslipidemia. It is also now recognised that insulin resistance may be the common link between obesity, impaired glucose tolerance or type 2 diabetes, dyslipidemia (high LDL cholesterol, low HDL cholesterol, high TAG concentrations), hypertension and impaired fibrinolysis which together have been called metabolic syndrome or syndrome X (Reaven *et al.* 1996).

Diet has an important role to play in the prevention of CHD, type 2 diabetes, the metabolic syndrome and insulin resistance (Hankey & Leslie, 2001; Fung *et al.* 2001; Joshipura *et al.* 2001; Hu *et al.* 2001b; Poulter, 2003). The most important aspect of diet seems to be to consume a diet that will provide enough energy to maintain a healthy body weight and avoid overweight. Of course, regular physical

activity is a very important part of maintaining a healthy body weight. However, as well as the overall energy intake, the composition of the diet has also been shown to be important, and current dietary guidelines recommend a reduction in total and saturated fat intake and increasing carbohydrate intake, especially complex carbohydrates (Department of Health, 1991; Department of Health, 1994; FAO/WHO, 2003)

However, as dietary fat is reduced and is replaced with carbohydrate, the desired reduction in LDL cholesterol is often accompanied by abnormal lipid concentrations such as an increase in TAG concentrations and a reduction in HDL cholesterol concentration (Jeppesen *et al.* 1997; Mensink & Katan, 1992), which has been shown to be associated with increased risk of CHD (Austin *et al.* 1999).

However, not all carbohydrates have the same effects on health (Parks & Hellerstein, 2000) and it may be that carbohydrates that are broken down into their constituent sugars very quickly and have consequent effects on raising blood glucose and insulin levels are the problem. Glycaemic index (GI) is a physiological measure of the ability of carbohydrate foods to raise blood glucose levels (Jenkins *et al.* 1981). A food with a high GI produces a much larger area under the blood glucose curve after consumption compared with a low GI food of equivalent carbohydrate content. The glycaemic load (GL) is a measure of both the GI and the amount of carbohydrate contained in a food. Examples of foods with low GI include pulses, wholegrain foods such as rye bread, and porridge; and examples of high GI foods include white bread, mashed potato and cornflakes. When foods with similar amount of carbohydrate are compared, there is a gap of (up to 10 fold differences) in their glycaemic effects (Foster-Powell *et al.* 2002; Brand-Miller & Holt, 2004).

There is currently much interest in the concept of GI and in the ability of low GI diets to reduce risk of chronic disease. There is evidence available from a number of large epidemiological studies that have shown that the dietary GI and GL may be important in the prevention of CHD (Liu *et al.* 2000) and type 2 diabetes (Salmeron *et al.* 1997a; Salmeron *et al.* 1997b) and in the control of risk factors for these diseases (Frost *et al.* 1999; Ford & Liu, 2000; Liu *et al.* 2001; Brand-Miller *et al.* 2003). However, not all studies have shown that GI is important in risk of chronic disease (van Dam *et al.* 2000).

Furthermore, it seems that there is no general agreement about the importance of GI on human health, nutrition and disease prevention (Ludwig & Eckel 2002). In fact while a number of major organisations (European Association for the Study of Diabetes, EASD, 2000; Canadian Diabetes Association, 2000; FAO/WHO, 2003; Diabetes UK, 2003) encourage the use of the GI concept, there are also a number of large organisation who do not accept or encourage the use of GI in dietary management (American Heart Association, 2000; American Diabetes Association 2001).

The objectives of the studies in this thesis were:

1. To investigate the effect of advice to increase carbohydrate intake as part of dietary advice to follow the UK dietary guidelines on some metabolic risk factors for CHD in healthy free-living postmenopausal women (Chapter 3).
2. To assess habitual dietary intake, GI, GL, anthropometric measurements and metabolic risk factors for CHD and type 2 diabetes (Chapter 4).

3. To examine the relationships between GI, GL and anthropometric characteristic and metabolic risk factors in offspring of patients with type 2 diabetes and in control subjects (Chapter 4).
4. To examine the influence of high carbohydrate, isocaloric, high and low GI diets for three days on metabolic parameters in the fasted state in healthy male subjects (Chapter 5).

6.2 Summary of findings

6.2.1. *Study to investigate the effect of advice to increase carbohydrate intake as part of dietary advice to follow the UK dietary guidelines on metabolic risk factors for CHD in healthy free-living postmenopausal women*

After the menopause, women lose the protective effects that the reproductive hormones have on lipid metabolism, and their risk of CHD is increased (Rich-Edwards *et al.* 1995; Jeppesen *et al.* 1997). There are no specific dietary guidelines for the prevention of CHD for postmenopausal women, and the role of diet and in particular, carbohydrate intake has not been well studied in this vulnerable group. In this study, effect of increasing carbohydrate intake, with emphasis on starch, as part of dietary advice to follow the current dietary guidelines on some metabolic risk factors for CHD was examined in a group of postmenopausal women.

The main finding of this study showed that there was a significant reduction in body mass index (BMI) after the four-week intervention. Subjects appear to have followed the dietary advice given as they reported significantly reducing their total daily

energy, fat and non-milk extrinsic sugar (NMES) intake, and significantly increasing their total carbohydrate, starch and non-starch polysaccharide (NSP) intake. Subjects also significantly increased their dietary GI and GL during the intervention. There was an adverse effect on fasting plasma lipids including an increase in fasting TAG, and a decrease in HDL cholesterol concentrations. A number of correlations were carried out, and they showed a protective effect of complex carbohydrates and starch and an adverse effect of simple sugars on fasting plasma lipid concentrations. Subjects reported increasing their consumption of fruit and vegetables, and there was a significant increase in the 'antioxidant power' of plasma as measured by FRAP. This appears to have mostly been associated with an increase in fruit intake.

The dietary intervention was associated with a significant reduction in BMI, and this was likely to be due to the fact that the subjects reduced their energy intake as a result of following advice to reduce fat intake. However, even though subjects were asked not to change their lifestyle or physical activity levels (other than diet) during the study, activity levels were not measured or recorded, therefore it is not definite that the observed reduction in BMI was not due to an increase in physical activity. The advice to increase carbohydrate intake was also associated with significant increases in dietary GI and GL, which from the literature would be associated with increased risk of CHD (Liu *et al.* 2000) and type 2 diabetes (Salmeron *et al.* 1997a and b) and a worsening of metabolic risk factors (Frost *et al.* 1999; Ford & Liu; 2000; Liu *et al.* 2001) for these diseases. The advice given in this intervention study was associated with adverse effects on TAG and HDL cholesterol, however, the advice to increase fruit and vegetable intake appears to have had a positive effects on the 'antioxidant power' of plasma. In conclusion, although the dietary advice given

to these women appeared to have some important positive effects on health, namely on weight loss and on the 'antioxidant power' of plasma, adverse effect on plasma lipids were also observed. Thus, the findings of the present study show that more research is needed to develop more appropriate dietary advice for reducing not only some but all aspects of risk of CHD in postmenopausal women.

In this study, it was seen from the correlations that were carried out that simple sugars appeared to have a more adverse effect on plasma lipids than starch. Due to this finding, it was decided that it would be very interesting to look at relationships between GI, which is a physiological measure of the blood glucose raising ability of carbohydrate containing foods which might reveal the real effects of carbohydrates on plasma lipids and other metabolic risk factors in data that had already been collected on offspring of patients of type 2 diabetes and control subjects.

6.2.2. *Relationships between dietary glycaemic index and metabolic parameters in offspring of patients with type 2 diabetes and control subjects.*

Type 2 diabetes is a major public health concern as it increases risk of CHD and other complications and its rates are increasing worldwide. Offspring of patients with type 2 diabetes have an increased risk of developing the condition, and this is thought to be due to both genetic and lifestyle factors such as diet and physical activity. Improving diets has been shown in a number of large intervention studies (Pan *et al.* 1997; Uusitupa *et al.* 2000; Tuomilehto *et al.* 2001; Knowler *et al.* 2002) to substantially reduce the risk of developing type 2 diabetes in high risk groups. Furthermore, a number of cohort studies (Salmeron *et al.* 1997a; Salmeron *et al.*

1997b) have shown that diets with low GI and GL may be protective against the development of type 2 diabetes.

The main findings of this case control study on offspring of patients of type 2 diabetes (offspring) and control subjects showed that there were no differences in habitual dietary intake, GI or GL between the groups. Only a small proportion of the offspring and control subjects followed the dietary guidelines. A number of recent large intervention studies (Pan *et al.* 1997; Uusitupa *et al.* 2000; Tuomilehto *et al.* 2001; Knowler *et al.* 2002) have shown that improving diet can substantially reduce risk of developing type 2 diabetes in people who have a high risk. However, the offspring subjects in this study did not seem to be aware of their higher risk of developing diabetes, as they were not found to be following a healthier diet than the control subjects. Offspring were found to have greater levels of adiposity with a greater proportion of the offspring subjects having a BMI > 27.5 $kg.m^2$. Female offspring were found to have a significantly higher waist to hip ratio ($P = 0.036$), and a higher waist circumference ($P = 0.063$) and BMI ($P = 0.083$) compared with female control subjects. Offspring were found to have a poorer metabolic profile and appeared to be significantly more insulin resistant compared with control subjects with significantly higher fasting insulin ($P = 0.049$) and higher $HOMA_{IR}$ ($P = 0.052$), and significantly lower HDL cholesterol concentrations ($P = 0.011$).

A number of correlations were carried outto study relationships between the glycaemic quality of the diet and anthropometric characteristics and metabolic risk factors. Dietary GI and GL were not found to be directly associated with any of the metabolic parameters measured in the study, but GI was positively correlated with waist circumference ($P = 0.039$) and waist to hip ratio ($P = 0.043$), and these

measures of adiposity (i.e. waist circumference and BMI) were significantly correlated with many of the metabolic parameters measured in the study. Thus, while the glycaemic quality of the diet did not appear to directly influence metabolic risk factors, the results do support the idea that they influence metabolic risk factors through their affect on adiposity, and in particular central adiposity. Of course this study was not able to show this for certain. In support of these findings there are many large epidemiological studies (Salmeron *et al.* 1997a; Salmeron *et al.* 1997b; Frost *et al.* 1999; Liu *et al.* 2000; Liu *et al.* 2001) that have shown that GI may be important for risk of type 2 diabetes.

Thus, this cross-sectional study provided some idea that the glycaemic quality of the diet could have an effect on metabolic risk factors, which is supported by epidemiological studies. There were very few intervention studies that had been carried out in healthy individuals, of either short or longer-term. It was decided that it would be interesting to see if the benefits of reducing fat intake (i.e. cholesterol lowering) could be maintained, and the adverse effects associated with increasing carbohydrate intakes (increasing TAG and reducing HDL cholesterol) that were observed in the first study on postmenopausal women, could be avoided if dietary fat was replaced with carbohydrates of low GI. For this reason, it was decided to carry out a high and low GI intervention study on healthy individuals using a high-carbohydrate, short-term study model (Koutsari *et al.* 2000), which had been shown to be a good model for studying effects on plasma lipids.

6.2.3._ *The influence of high carbohydrate, isocaloric, high and low GI diets for 3 days on metabolic parameters in the fasted state in healthy male subjects.*

This was a randomised, cross-over study in which subjects recorded their habitual diet and had a baseline blood sample taken and then followed high carbohydrate, high and low GI diets for three days with a washout period between. The results of the study showed that the low GI diet had some beneficial effects in that it reduced total and LDL cholesterol. Both high and low GI diets had adverse effects on TAG and HDL cholesterol concentrations but this was probably due to the fact that the diets were very high in carbohydrate (70% energy intake) as high carbohydrate diets have been shown to have this effect in many other studies. TAG concentrations were found to be higher after the low GI diet, which was surprising, as studies in the literature have generally reported that low GI diets either have no effect or reduce TAG concentrations. In this study, this could possibly be explained by the fact that the low GI diet was higher in sugars compared with the high GI diet, and it has been reported that sugars have an adverse effect on plasma lipids (Parks & Hellerstein, 2000). These results are interesting and suggest that the overall carbohydrate and sugar content of the diet have a more important influence on plasma lipids and other metabolic parameters than the glycaemic quality of the diet. Of course, it should be remembered that the intervention diet used in this study was high in carbohydrate and for this reason, these results may not be applicable to the general population who would probably not have this much carbohydrate in their diet. Many studies have shown beneficial effect of low GI diets (Salmeron *et al.* 1997a; Salmeron *et al.* 1997b; Frost *et al.* 1999; Liu *et al.* 2000; Ford & Liu, 2000; Liu *et al.* 2001). Also, diets of low GI tend have other healthy characteristics, for example, they tend to contain more non-starch polysaccharide, antioxidants and other micronutrients, and

tend to be lower in fat. All of these dietary aspects have been shown to have protective health effects. Finally, it should be remembered that his was an experimental, mechanistic type study and future studies should look at the effects of glycaemic index diets in diets with a more realistic carbohydrate content (50% energy) which would have more relevance to real life situations and to public health.

Before concluding this thesis, a few methodological points will be considered. First, it would have been better to assess lipids in the postprandial as well as the fasted state. It is generally agreed (Zilversmit, 1979; Parks *et al.* 2002) that disease risk is better predicted by a postprandial test, especially in the case of TAG, as most individual are in the postprandial state for 16-18 h/d and only in the fasted state for about 6 h in the middle of the night. Unfortunately, collection of postprandial blood samples was not possible in this study.

An important issue when studying the GI and GL is that the calculation of GI and the GL of the diets was performed using published glycaemic index tables (Foster-Powell *et al.* 2002) rather than actually measuring the GI of the foods or meals or diets themselves. Throughout the world there are just 15 laboratories that have been approved to measure that actual GI of the foods (Foster-Powell *et al.* 2002). This is a very difficult issue when considering the many variations in foods, food composition and food preparation all of which may affect digestibility and glycaemic response. It would be impossible to measure GI directly for each food in a study, but if GI and GL are to be used for scientific research then much more comprehensive tables are needed to cover the huge range of foods and country specific data may be needed.

Each of the studies in this thesis involved dietary assessment methods and there are well known errors associated with these methods including reporting error, difficultly in assessing real habitual diet, errors associated with using the food tables. It must be admitted that the use of the cut-offs for exclusion of under and over-reporters are limited and imperfect as they only exclude individuals who have reported biologically implausible energy intakes and do not identify individuals who mis-report to a lesser degree. The methods for dealing with these are described in the relevant chapters.

6.3 Conclusion

In both the intervention studies described in my thesis, adverse effects of increasing carbohydrate intakes on plasma lipids were found. In the first study, healthy free-living postmenopausal women were simply advised to increase their carbohydrate intake to the level recommended by the current dietary guidelines (50-55% energy) (Chapter 3). The second was an experimental, more mechanistic type, high carbohydrate (70% energy) intervention study in which healthy male subjects were provided with all the foods and give detailed instructions on diet (Chapter 5). The adverse effects on plasma lipids include an increase in TAG and a reduction in HDL cholesterol concentration, and are associated with in increased risk of CHD (Austin *et al.* 1999). In my third study (Chapter 5), even when subjects were advised to increase carbohydrate intake and the carbohydrate food were low GI, adverse effects on these lipid parameters were still observed. Thus, it seems that increasing carbohydrate intake even if the carbohydrate is low GI still has adverse effects on plasma lipids. Of course, the particular characteristics of the low GI diet used in our study, which was high in carbohydrate and sugar may explain this.

While there are many epidemiological studies that have reported protective effects of low GI diets on risk of CHD and type 2 diabetes and these have been referred to numerous times in this thesis, in fact very few intervention studies have been carried out looking at the effect of GI on metabolic risk factors in healthy individuals. Two meta-analyses on the effects on health effects of GI have recently been published, one on mainly diabetic individuals (Opperman *et al.* 2004) showing that low GI diets significantly reduced indicators of glycaemic control, total and LDL cholesterol but no significant effects on HDL cholesterol or TAG were reported. The other on individuals with at least one risk factor for CHD (Kelly *et al.* 2004) reported weak evidence of an effect of low GI diets on total cholesterol but no evidence of an effect on LDL or HDL cholesterol, TAG, fasting glucose or insulin levels.

GI is a measure of the blood glucose raising ability of carbohydrate foods and in general low GI diets are made up of carbohydrate foods that release glucose slowly into the blood stream. It is by this mechanism that it is hypothesised that low GI diets would have a beneficial effect on glycaemic control, blood, lipids and other metabolic parameters. However, one problem with GI is that foods that are high in fructose such as ripe sweet fruit and foods sweetened with high fructose corn syrup have low GI values. This is because fructose does not have the same effect on raising blood glucose and stimulating insulin secretion and causes a smaller blood postprandial insulin secretion compared with glucose containing foods (Elliott *et al.* 2002). However, the problem is that fructose still does have adverse effects on TAG levels (Jeppessen *et al.* 1995). Thus, this issue may be a major problem with the use of GI and for this reason, it may be better to go back to the idea of advising people to eat more slowly digestible carbohydrates, for example beans pulses and whole grains, rather than low GI diets which could still contain a lot of fructose-containing

foods which may mask the positive effects of the low GI diet on lipids and especially TAG.

If we are to give beneficial dietary advice to the general public then we need to give advice based on good science and using good communication. Most people do not understand which foods are high in sugar but have a low GI and, as seen in study one, the response to advice to increase carbohydrate intake often resulted in increased sugars. We need to do more research to find develop better and more specific dietary guidelines and to educate the public so they can use them appropriately.

Chapter 7

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