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Factors influencing the effects of diet on insulin resistance and metabolic parameters in NIDDM

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FACTORS INFLUENCING THE EFFECTS OF DIET ON INSULIN RESISTANCE AND METABOLIC PARAMETERS IN NIDDM

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ABSTRACT

This is a randomised prospective controlled study on the effectiveness of two diet programs, one an orthodox high carbohydrate low fat diet (Control) and the other a high monounsaturated fat diet (HMUFA) (also containing n-3 α-linolenic acid) on insulin resistance, serum lipids and other variables in 50 adult patients with non-insulin dependent diabetes mellitus (NIDDM) during one year. Patients were randomly allocated to the two dietary groups. The HMUFA group were given a diet program (using canola and olive oils and margarines, nuts, farmers best milk, avocado) to maintain body weight, aimed at providing 40% of energy intake as carbohydrate (CHO) and 38% as fat (21% monounsaturated). The Control group were given a diet program containing 50% of energy intake as CHO and 24% as fat. A 4 day food record was analysed every 3 months. Insulin resistance was measured in a subgroup of 28 participants by the hyperinsulinemic euglycemic clamp technique. After 12 months the following changes had occurred. The HMUFA group had 34% of energy intake as fat (16% monounsaturated, 10% saturated), and the Control group 31% (13% and 11% respectively). The HMUFA group had significant reductions in systolic ($p=0.038$) and diastolic blood pressure (BP) ($p=0.009$), fasting plasma glucose ($p=0.026$), triglyceride ($p=0.050$), and significant increases in HDL-cholesterol ($p=0.011$) and insulin sensitivity ($p=0.005$), compared to Control group. In HMUFA mean BP fell from 135/83 to 127/73 in 12 months: there was no change in the Control group. The mean fasting blood glucose fell from 9.2 to 8.3 mmol/L in the HMUFA group, and rose from 8.5 to 9.5 mmol/L in the Control group. The mean insulin sensitivity rose significantly from 111 to 160 $mg.m^{-2}.min^{-1}$ in the HMUFA group, and fell from 130 to 91 $mg.m^{-2}.min^{-1}$ in the Control group. This study concludes that after one year the monounsaturated-enriched diet (containing also n-3 α-linolenic acid) program was associated with a better metabolic profile in NIDDM patients than a high carbohydrate low fat diet.

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Glossary of Abbreviations

BMI	Body Mass Index
CETPA	Cholesteryl Ester Transfer Protein Activity
CHO	carbohydrate
CVD	Cardiovascular disease
D.B.P	diastolic blood pressure
FBG	fasting blood glucose
HDL	High density lipoprotein
IDDM	Insulin dependent diabetes mellitus
IDL	Intermediate density lipoprotein
IDL-C	Intermediate density lipoprotein cholesterol
IDL-TG	Intermediate density lipoprotein triglyceride
IGT	Impaired glucose tolerance
LDL	Low density lipoprotein
MAP	mean arterial pressure
mono.	monounsaturated fat
NEFA	Non-Esterified-Fatty Acid
NIDDM	Non-insulin dependent diabetes
poly.	polyunsaturated fat
sat.	saturated fat
S.B.P	systolic blood pressure
TC	total cholesterol
TG	triglyceride
VLDL	Very low density lipoprotein
VLDL-C	Very low density lipoprotein cholesterol
VLDL-TG	Very low density lipoprotein triglyceride
W:H ratio	waist : hip ratio

PRESENTATIONS

This study was presented in the following events:

* Symposium of Biomedical Research in *Wollongong*, June 1995 - poster

* University of Wollongong Open Day, *Wollongong*, September 1995 - poster

* European Association for the Study of Diabetes, *Vienna*, September 1996 - oral

* Postgraduate Research Student Day, *Wollongong*, September 1996 - poster

* Australian Diabetes Society, *Sydney*, October 1996 - poster

CHAPTER ONE

INTRODUCTION

1.1 Background:

Diabetes mellitus is a common disorder in every part of the world. It is a chronic disorder characterised by fasting hyperglycaemia and insulin resistance (Nettleton, 1995).

Type II diabetes mellitus or non-insulin dependent diabetes mellitus (NIDDM) is certainly the most common type of diabetes, accounting for 80% to 90% of those diagnosed with diabetes (Henry, 1996). NIDDM is an important health concern because it predisposes to cardiovascular disease and chronic complications including retinopathy and neuropathy (Kannel et al., 1986; Coulston 1994). People with diabetes comprise one-third or more of those who have had a renal transplant (Jenkins et al., 1989). Because NIDDM is a slowly progressive disease, the number of undiagnosed individuals is nearly the same as the number who have been diagnosed (Beebe et al., 1991).

Every 15 minutes in Australia someone is diagnosed with diabetes. Three to four per cent of the Australian population have diabetes and it is claimed that another 3% have undiagnosed diabetes (Diabetes Australia). In a country of 17 million people, diabetes ranks fifth after heart disease, stroke, musculo-skeletal disease and cancer as a cause of death (Turtle et al., 1994), and Australian Aborigines suffer the 4th highest rates of NIDDM in the world (McCarty et al., 1996). If grouped together with vascular disease, the combination is the most common cause of death in Australia (Turtle et al., 1994). In 1990, approximately 350,000 Australians had

"diagnosed" diabetes and an estimated 300,000 Australian had "undiagnosed" diabetes. Diabetes is likely to affect 900.000 Australians by year 2000 and 1.15 million Australians by year 2010 (McCarty et al., 1996). It has been estimated that all diabetes accounts for over 8 per cent of the acute hospital budget (Marks, 1996). The total cost of diabetes likely exceeds $1 billion annually, or about $2,800 per diagnosed case (McCarty et al., 1996).

A similar pattern has been in other countries - a worldwide explosion in the frequency of diabetes and the morbidity resulting from its complications. At present, 45.8% of the direct annual costs of diabetes are spent in hospital care with a further 22.6% in nursing home care, leaving only some 22% of diabetes costs directed towards medical care, health personnel, drugs, etc. At least two-thirds of this cost is related to NIDDM and its complications (Turtle et al., 1994).

It is agreed that NIDDM is caused by a combination of genetic and environmental factors (Kahn, 1994; Taylor et al., 1994; Ghosh and Schork, 1996).

Among the environmental variables diet is considered as the major factor, influencing the condition and treatment of NIDDM patients (Storlien et al., 1991). It is thought that the risk of developing NIDDM is influenced with diet and can be controlled to a large extend by food choices (Nettleton, 1995).

1.2 Diet:

Nutrition therapy is an important component of a successful management program for NIDDM. The two main aims of diet therapy for people with diabetes are the improvement of glycaemic control, and reduction of the risk of coronary heart disease by optimising serum lipid levels. The American Diabetes Association

(ADA) (1994) stipulates that nutritional therapy for individuals with NIDDM should be used to achieve nominated blood glucose, lipid and blood pressure goals.

Dietary recommendations for treatment of diabetic patients issued by national and international diabetes associations consistently emphasise the need to increase carbohydrate consumption (substituting for energy from fat). However, these recommendations have been questioned on the basis of growing evidence that, in people with both IDDM and NIDDM, a high-carbohydrate diet does not offer any advantage in terms of glycemic control and plasma lipid compared with a diet containing liberal unsaturated fat. In recent years there have been concerns about recommending high-carbohydrate diets because of potentially harmful effects of such diets on plasma lipoproteins, ie. an increase in plasma TG and lowering HDL concentrations (Riccardi and Rivellese, 1991), which ultimately could increase the risk of developing atherosclerosis (Mensink and Katan, 1987; Garg et al., 1988; Mata et al., 1992; Campbell et al., 1994).

Studies have also shown that adherence to the recommended low fat high carbohydrate diet over an extended period of time is sub-optimal (Campbell et al., 1989). All of these issues are quite disturbing considering the importance of diet in the treatment of NIDDM.

The use of fat as a replacement source of energy for some of the carbohydrate has been suggested (ADA, 1997). Both saturated fat (Simopoulos, 1994) and polyunsaturated fat (Grundy and Denke, 1990; Grundy, 1991) have been associated with factors which can increase the risk of developing cardiovascular disease by lowering HDL-C especially when consumed in large quantities. The use of monounsaturated fat as a replacement source of energy for some of the

carbohydrate has been under investigation in recent years (Garg et al., 1992; Campbell et al., 1994).

1.3 Insulin resistance:

Insulin resistance is thought to provide the pathophysiological basis for the increased risk of cardiovascular morbidity in NIDDM (Nosadini et al., 1993). During the last 20 years several studies applying different techniques to evaluate the degree of insulin resistance have repeatedly shown that the vast majority of NIDDM patients are insulin resistant (Ferrannini et al., 1991; Seely and Olefsky, 1993; Laakso, 1993). The presence of insulin resistance is characterised by multiple defects in lipid metabolism (discussed in chapter 2 of this thesis). These defects lead to abnormalities in lipid composition and lipoprotein concentration which could increase the risk of coronary heart disease (Zavaroni, 1993; Inchiostro et al., 1994) and morbidity in NIDDM (Laakso et al., 1993; Nosadini et al., 1993). Diet is also considered an important environmental factor influencing insulin sensitivity (Borkman et al., 1991). There is also growing evidence that there may be a link between the dietary fat quality and the risk of developing peripheral insulin resistance (Vessby et al., 1994).

1.4 Rationale of the study:

In view of the uncertainty on optimum dietary prescription for NIDDM, the current study was established, to contract the two leading dietary regimes in free living people with NIDDM over the course of a year.

1.5 Aims of the study:

The major aims of the study are to test, in a one-year randomised prospective intervention two null hypotheses, which, stated in the alternate form, are:

1) A diet enriched with monounsaturated fatty acid and with an increased n-3 α-linolenic acid content has superior effects on glycemia, plasma lipids, and insulin resistance (ie. lower plasma glucose, insulin, and triglyceride after a 12-hour overnight fast, higher plasma HDL-cholesterol, and lower insulin resistance) in patients with NIDDM, compared to a diet low in fat with a high contents of complex carbohydrate.

2) A diet enriched with monounsaturated fat and n-3 α-linolenic acid is more palatable and more acceptable for patients with NIDDM than the low-fat high complex carbohydrate diet.

1.6 Research objectives:

The research objectives are to find out if:

(a) There are any significant differences after 3 months and 12 months in metabolic parameters in people with NIDDM on a modified fat diet (enriched with monounsaturated fatty acid and with an increased n-3 α-linolenic acid) compared with a similar group on a low fat high complex carbohydrate diet.

(b) There are differences in these parameters between status at the beginning of the study and at 3 and 12 months, in each diet group.

Study variables measured at 0, 3, and 12 months include:

1. Anthropometric measurements: Weight, height, waist and hip circumference, skinfold thickness at several sites (triceps, biceps,

subscapular, suprailiac areas), and variables derived from these measurements;

2. Blood pressure (systolic and diastolic);

3. Plasma glucose and insulin after fasting, glycosylated haemoglobin (HbA1c), and C-peptide;

4. Lipids and lipoproteins: plasma cholesterol, triglyceride, HDL cholesterol, LDL cholesterol, apolipoproteins B and A-I, VLDL and IDL cholesterol and triglyceride, and Cholesteryl ester transfer protein activity;

5. Insulin sensitivity (in a sub-group);

6. Indirect calorimetry (in a sub-group);

7. Food and nutrient consumption patterns.

(c) Qualitative aspects relating to dietary changes (difficulties in adopting dietary recommendations etc.) at 12 months.

1.7 Significance of the study:

This study is as an investigation into the feasibility and effectiveness of a diet containing liberal oils, rich in monounsaturated fatty acids and with an increased n-3 α-linolenic acid content (canola and olive in the form of oil; margarine; mayonnaise; nuts, avocado, and farmers best milk) in patients with NIDDM, compared with the orthodox low fat high complex carbohydrate diet. The results may be of practical use in the treatment of diabetes.

Previous studies in this area have often used prepared diets or liquid formula in metabolic wards, sometimes with extremes of fat or carbohydrate intake outside the limits of practicable intakes in daily life (Howard et al, 1991). Very few studies

have investigated the use of a diet containing liberal monounsaturated fatty acids in an outpatient setting, with no dietary supplementation to the subjects.

Research into the effectiveness of a fat-modified diet in individuals with NIDDM on a long term basis in real life situations is extremely limited. All previous such studies have extended over only a few months. In this study we are examining the effect of this for a period of 12 months.

1.8 Summary:

Although dietary treatment is accepted as an integral part of the management of NIDDM (Milne et al., 1994), opinion on the optimum dietary regimen for individuals with NIDDM remains unsettled, even though no other disease in medical history has been treated by dietary means more intensively (Marmot, 1992). This study is a contribution to the solution of this problem.

CHAPTER TWO

LITERATURE REVIEW

2.1 Diabetes mellitus:

2.1.1 Introduction:

Diabetes is a chronic disorder characterized by fasting hyperglycaemia and insulin resistance (McDonald and Roberts 1990; Nettleton, 1995). The development of diabetes can be influenced by a number of factors, hereditary, environmental, and hormonal (Bloom & Ireland 1992).

Several types of diabetes are described: Type I or insulin-dependent diabetes mellitus (IDDM), type II or non-insulin dependent mellitus (NIDDM), malnutrition-related diabetes mellitus (MRDM), gestational diabetes mellitus (GDM) (Bloom & Ireland 1992), and maturity-onset-type diabetes of the young (MODY) (Cox et al., 1992).

Type II diabetes or NIDDM accounts for 80% to 90% of those diagnosed with diabetes (Henry, 1996). It occurs most frequently in middle aged or elderly individuals (Zeman, 1991; Williams, 1994). The diagnosis is often made through routine examination, fasting blood sugar, and sometimes glucose tolerance test (GTT) (Bloom & Ireland 1992). Individuals with NIDDM are often obese (Griver and Henry, 1994) and characteristically display hypertriglyceridaemia and low HDL cholesterol levels (Stern and Haffner, 1991).

NIDDM is an important health concern because it predisposes to cardiovascular disease and other chronic complications including retinopathy and neuropathy

(Kannel et al, 1986; Coulston, 1994). NIDDM affects about 85 per cent of the 3 per cent of Australian with the diagnosis of diabetes (Nutbeam et al., 1993). People with diabetes make up one-third or more of the renal transplant population (Jenkins et al., 1989). Because it is a slowly progressive disease, the number of individuals with undiagnosed NIDDM is though to be nearly the same as the number who have been diagnosed (Beebe et al., 1991).

2.1.2 Pathogenesis of diabetes in NIDDM:

There are at least two fundamental defects in the pathogenesis of NIDDM which are caused by a combination of genetic and environmental factors (Kahn, 1994; Taylor et al., 1994; Ghosh and Scarbohydraterk, 1996). One is a decreased ability of insulin to act on peripheral tissues to stimulate glucose metabolism or inhibit hepatic glucose output, known as insulin resistance (Kolterman et al., 1981; DeFronzo et al., 1992). The other is the inability of the endocrine pancreas to completely compensate for this insulin resistance (Orci et al., 1990; Porte, 1991). (Figure 2.1)

There is a general agreement that the majority of patients with NIDDM are insulin resistant, and that the development of insulin resistance is an early event in the natural history of the disease (Bogarduus, 1993; Daly, 1994; Simopoulos, 1994a).

The causes of NIDDM are unclear, but it is generally accepted that they are likely to be multiple, including polygenic and environmental factors. The contribution of genetics to the etiology of NIDDM is illustrated by studies of identical twins. Barnett et al. (1981) has reported that in 53 identical twin pairs with at least one affected subject, 91% of the co-twins developed NIDDM. The co-twins in the five discordant pairs had mild glucose intolerance and abnormal insulin resistance, indicating that they might ultimately progress to overt disease and push the concordance rate to 100%. In contrast, only 54% of IDDM twin pairs were

concordant for IDDM. Despite the high concordance rate in twins, it is obvious that NIDDM is not simply the result of a single gene defect, because the incidence of NIDDM in first- and second-degree relatives is lower than one would expect in such a case (Barnett et al., 1981; Warram et al., 1990).

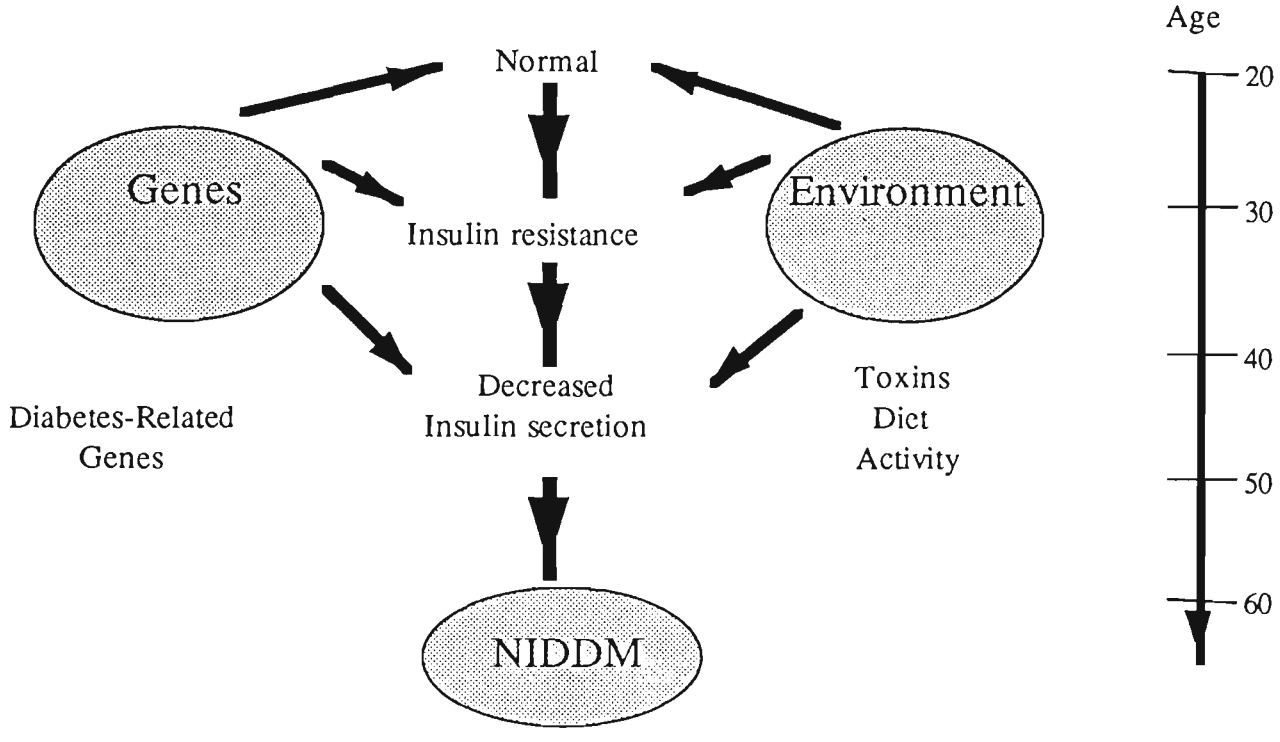

Figure 2.1: An overview of the natural history of NIDDM and the impact of genes and the environment in this process (Kahn, 1994). This schema is greatly simplified; for instance, the development of insulin resistance is accompanied by increased insulin secretion. Insulin secretion is decreased only when pancreatic β-cell *failure supervenes.*

2.1.2.1 Polygenic mechanisms:

Many studies are in progress on the genetics of NIDDM, but relatively few studies have adopted an interdisciplinary approach to examine the genetic and environmental factors (Ghosh and Scarbohydraterk, 1996).

In rare cases single genes alone can lead to NIDDM, but in the most cases it seems that NIDDM is results of combined defects of impaired β-cell function and insulin action, either of which can in principle have a genetic or environmental basis (McCarthy and Hitman, 1993).

Recently mutations of the glucokinase gene, leading to NIDDM, have been described. It appears though that this candidate gene is not responsible for the majority of NIDDM cases, and it is generally believed that NIDDM is in most cases polygenic. In addition, environmental factors appear to be needed for NIDDM to be manifest (Laakso, 1993; Armstrong et al., 1995).

The search for genetic determinants of NIDDM is complicated enormously by the fact that the basic biochemical defects underlying glucose intolerance have not been established (McCarthy and Hitman, 1993).

The search for genes that might be involved in the etiology of NIDDM has not yielded consistent results (Laakso, 1993; Ghosh and Scarbohydraterk, 1996). For instance, it is not clear what determines which patients with mutations in the insulin or glucokinase genes will become diabetic (Taylor et al., 1994).

2.1.2.2 Proposed environmental mechanisms:

Environmental factors apparently play a role in unmasking the underlying genetic susceptibility, a "thrifty" diabetic genotype that probably developed from primitive times for survival. This theory (the thrifty genotype) is that diabetes may be associated with genetic modifications for survival during periods of food unavailability. As food supplies become more plentiful, negative aspects of the diabetic trait began to appear. For example, the Pima Indians in Arizona were

probably not prone to diabetes in earlier times, when the ready ability to store food as fat was a survival trait. Now with the "progress" of civilization they readily become obese and half of the adults have NIDDM, probably the highest reported rate of this type of diabetes in the world (Bennett, 1990; Hales and Barker, 1992; Williams, 1994; Stern, 1996). Australian Aborigines are another groups who have a major susceptibility to NIDDM.

The evidence suggests that groups such as these have a genetic susceptibility to NIDDM, but the development of NIDDM is triggered by environmental factors like over-nutrition and little physical exercise (Helmrich et al., 1991; Taylor et al., 1994; Williams, 1994; Knowler and Narayan, 1994).

2.1.2.3 Lipotoxic theory:

Unger (1995) has suggested that increased tissue levels of fatty acyl CoA cause β-cell abnormalities in non-diabetic obesity and finally result in obesity-dependent diabetes. Non-diabetic obesity in Zuker rats is characterised by hypersecretion of insulin at normal fasting and subfasting glucose concentrations. This is a result of β-cell hyperplasia and increased low K_m glucose usage and oxidation. These abnormalities (hyperinsulinemia, β-cell hyperplasia) enhanced bromodeoxyuridine incorporation, and the increased low K_m glucose usage can be induced by culturing normal islets with 2 mmol/l free fatty acids (FFAs).

Once obese Zucker diabetic fatty rats become diabetic, glucose stimulated insulin secretion (GSIS) is absent and β-cell GLUT2 reduced. Islet triglyceride (TG) content is increased 10-fold, probably reflecting increased FFA delivery (plasma FFA levels > 1.5 mmol/l) beginning about 2 weeks before the onset of diabetes. These β-cell abnormalities, GSIS loss, GLUT2 loss, and TG accumulation, are

prevented by reducing plasma FFAs, either by caloric restriction or by nicotinamide injection (Unger, 1995).

Therefore, it is proposed by Unger (1995) that in uncomplicated obesity, increased lipid availability (FFA levels < 1.5 mmol/l) induces both hyperinsulinemia and insulin resistance in parallel fashion, thereby maintaining normoglycemia. A further increase in substrate overload impairs β-cell compensation for insulin resistance and hyperglycemia appears.

Vessby et al. (1994) investigated the risk of developing NIDDM among 50-years old men during a 10-years follow-up. They found that there were highly significant differences in the initial health survey between the fatty acid composition in serum in subjects who remained normoglycemic (n=1,753) and in those who later developed NIDDM (n=75). The main differences were that the latter had higher proportions of saturated fatty acids and palmitoleic acid (16:1ω-7), a low proportion of linoleic acid (18:2ω-6), and a relatively high content of γ-linolenic (18:3ω-6) and dihomo-γ-linolenic (20:3ω-6) acids in the serum cholesterol esters.

Although there is a possibility that genetically determined differences in the enzymatic handling of fatty acids in the body may contribute to explaining these findings by modulating both the fatty acid composition and insulin sensitivity (Borkman et al., 1993), there is a considerable possibility that the fatty acid composition in serum and / or cell membranes could influence the development of NIDDM and that dietary factors could play a role in this procedure (Vessby et al., 1994).

In summary, NIDDM is a disease with a slow onset and uncertain pathogenesis in which both genes and the environment are thought to play a critical role (Kahn, 1994).

2.2 Insulin resistance:

2.2.1 Introduction:

There is increasing evidence that insulin resistance is an important biological variable which potentially influences the development of a broad range of human disorders (Reaven, 1988). Insulin resistance is defined as a subnormal biologic response to a given concentration of insulin (Moller and Flier, 1991).

As insulin resistance is a risk factor for coronary artery disease, measuring insulin sensitivity in those at high risk can be beneficial (Laakso et al., 1993; Simopoulos, 1994). Although we can not do this easily, the measurement of sensitivity of the tissues to insulin would be desirable as an indication of the prevalence and/or progress of metabolic disease, and to evaluate the effectiveness of specific therapies (Bergman et al., 1987).

2.2.2 Factors influencing insulin resistance:

Insulin sensitivity has been shown to be influenced by genetic (Lillioja et al., 1987; Moller and Flier, 1991) and environmental factors (Walker and Alberti, 1993; Simopoulos, 1994a). These include primary inherited abnormalities of insulin action, and the effects of secondary metabolic changes, in particular chronic hyperglycemia and hypertriglyceridaemia, which are features of established NIDDM (Despres and Marette, 1994; Armstrong et al., 1996). Although several candidate genes have been identified, in only a small proportion of patients can insulin resistance be ascribed to genetic defects (Garg and Haffner, 1996).

Insulin resistance is frequently associated with common metabolic abnormalities, including hyperinsulinemia, obesity, hypertension, and dyslipidemia and as a complication of these, atherosclerotic vascular disease (DeFronzo and Ferrannini, 1991; Park, 1993; Durrington, 1995; Walker, 1995; Shinozaki et al., 1996; Stern, 1996).

During the last 20 years studies applying different techniques to evaluate the degree of insulin resistance have repeatedly shown that the vast majority of NIDDM patients are insulin resistant (Ferrannini et al., 1991; Seely and Olefsky, 1993; Laakso, 1993). Studies on individuals with a positive family history of diabetes in first degree relatives have suggested that insulin resistance may have a genetic basis (Eriksson et al, 1989). In Pima Indians a genetic determinant of insulin resistance may be related to the fatty acid binding protein gene on chromosome 4 (Prochazka et al., 1992).

Hepatic insulin insensitivity appears to contribute relatively little to overall measured insulin sensitivity (Bell et al., 1986; Firth et al., 1986). DeFronzo et al. (1985) showed that during a hyperinsulinaemic euglycemic clamp approximately 85% of intravenously administered glucose was taken up by peripheral tissues (almost entirely muscle). As hepatic glucose production appeared to be reduced to zero under the conditions of the clamp, it was deduced that peripheral tissue insulin insensitivity was the predominant lesion in NIDDM (DeFronzo et al., 1985; Armstrong et al., 1996).

2.2.3 Insulin resistance syndrome (IRS) and metabolic abnormalities:
Much attention has recently been focused on the insulin resistance syndrome, also referred to as `syndrome X'or `plurimetabolic syndrome' (Pozza et al., 1993).

The development of IRS in an individual is dependent, in addition to genetic factors, on many other modifying factors (Laakso, 1993). Clinically, the syndrome is characterised by metabolic and haemodynamic abnormalities. They include:

1- resistance to insulin-stimulated glucose uptake (Reaven, 1988; Nettleton, 1995; Stern, 1996);

2- glucose intolerance (Reaven, 1988; Moller and Flier, 1991; Alford, 1996);

3- hyperinsulinaemia (Reaven, 1988; Foster, 1989; Shafrir 1993; Borkman et al., 1993);

4- increased levels of VLDL-TG (Zavaroni, 1993; Reaven, 1988; Alford, 1996);

5- decreased levels of HDL cholesterol in both sexes (Stalder et al., 1981; Zavaroni et al., 1985; Laakso, 1993; Laakso et al., 1993; Alford, 1996; Stern, 1996) or only in men (Chaken et al., 1993);

6- hypertension (Daly and Landsberg, 1991; Reaven, 1991; Laakso, 1993; Nosadini et al., 1993; Shafrir 1993; Nosadini et al., 1994; Alford, 1996; Stern; 1996);

7- abdominal obesity (Stern, 1996).

Warram et al (1990) have reported that decreased glucose clearance may be demonstrated one or two decades before NIDDM is diagnosed. This decreased glucose clearance is accompanied by compensatory hyperinsulinemia, suggesting that an early defect in the development of NIDDM is in the peripheral tissue response to insulin and glucose.

Hyperinsulinemia may be recognized many years before the onset of NIDDM (Sicree et al., 1987; Saad et al., 1988; Haffner et al., 1990; Lundgren et al., 1990; Warram et al., 1990; Charles et al., 1991). Insulin resistance with hyperinsulinemia appears to be the earliest metabolic derangement, followed by hypertension. In animal models however these complications do not always appear, perhaps because of the short term of the experiments or short life span of the animals which do not allow the full expression of vulnerability (Shafrir, 1993).

It has been recognized for many years that insulin resistance in humans increases with age, independent of changes in total adiposity (DeFronzo, 1981; Rowe et al., 1983). However, there is growing evidence that the development of insulin resistance in NIDDM may be more closely related to abdominal adiposity (Kissebah et al., 1982; Krotkiewski et al., 1983; Ducimetiere et al., 1986; Park, 1993; Kohrt et al, 1993; Banerji et al., 1995) than age (Boden et al., 1993). Subjects with excess intra-abdominal (visceral) fat have more insulin resistance (Bjorntorp, 1989). Abdominal obesity is considered as a risk factor for the development of insulin resistance and its metabolically related abnormalities (Bjorntorp, 1990; Yamashita et al., 1996).

There is still controversy on the differential impact of fat accumulation on the development of insulin resistance; what are the comparative effects of intraperitoneal, retroperitoneal or subcutaneous abdominal adipose tissue? (Abate, 1996).

Chung et al. (1995) after studying glucose turnover and indirect calorimetry in 10 obese NIDDM and 10 lean NIDDM patients, found that obese NIDDM patients were significantly more insulin resistant than the lean group. They concluded that

obesity increases insulin resistance in NIDDM primarily by effects on glucose uptake rather than intracellular pathways of glucose metabolism. This finding has been supported by others as well (Bergman et al., 1987; Campbell and Carlson, 1993; Chung et al., 1995).

Castillo et al. (1994), noting that insulin resistance in obese subjects is not due to failure of insulin to reach its site of action but due to factors in the tissues themselves, suggested that an increased supply of amylin co-secreted with insulin during overfeeding and weight gain might impair insulin action (Castillo et al, 1994)

This relationship between insulin resistance, obesity, and NIDDM is very complex. Fujioka et al. (1987) have reported that in obese patients the fasting plasma glucose and area under the plasma glucose concentration curve after oral glucose loading (plasma glucose area) were significantly greater than in non-obese patients.

Paolisso et al. (1993) found that in the absence of other causes of insulin resistance, isolated hypercholesterolemia is associated with normal sensitivity to insulin in both liver and peripheral tissues, in young non-obese patients with primary familial hypercholesterolemia (FH).

Also the association between insulin resistance, raised triglycerides and low HDL cholesterol has been described in a number of studies (Taskinen, 1995). Mykkanen et al. (1995) demonstrated that high plasma insulin levels and insulin resistance were associated with low HDL cholesterol and high total triglyceride and VLDL triglyceride levels in both men and women.

In summary, the presence of insulin resistance in NIDDM is characterized by multiple defects like obesity and excess abdomen fat distribution. These defects

lead to abnormalities in lipid composition and lipoprotein concentration which could independently increase the risk of coronary heart disease (Zavaroni, 1993; Inchiostro et al., 1994) and morbidity in NIDDM (Nosadini et al., 1993).

2.2.4 The effect of diet on insulin resistance:

Among the environmental variables diet is considered as a major factor in the development of insulin resistance (Storlien et al., 1991). Recent studies indicate that there may be a link between the dietary fat quality and the development of peripheral insulin resistance (Vessby et al., 1994).

Borkman et al. (1991) compared the effect of a high carbohydrate (50% of energy intake) low fat diet and a high fat (45% of energy intake; predominantly saturated) low carbohydrate diet on insulin sensitivity, over 3 weeks for each diet in non-diabetic subjects. They suggested that practically achievable high carbohydrate diets do not enhance insulin sensitivity in non-diabetic subjects as the mean whole body glucose uptake was similar after each diet.

In another study, Borkman et al. (1993) reported that decreased insulin sensitivity is associated with decreased concentrations of polyunsaturated fatty acids in skeletal-muscle phospholipids, raising the possibility that changes in the fatty-acid composition of muscles modulate the action of insulin.

Studies in rats have demonstrated insulin resistance after feeding a diet high in fat (mainly polyunsaturated ω-6 type) or simple sugar (Kraegen, 1986; Storlien et al., 1986; Storlien et al., 1991; Ikemoto et al., 1995). Diets with high saturated fat content decrease insulin response in isolated rat adipocytes (van Amelsvoort et al., 1988, Chattaway et al., 1990; Storlien et al., 1993b; Simopoulos, 1994a). It has been suggested that the insulin resistance resulting from increased saturated fat

intake can be prevented by the inclusion of ω-3 polyunsaturated fatty acids in the diet, particularly docosahexaenoic acid (Storlien et al., 1993b).

Chen et al. (1988) investigated the effect of dietary changes on insulin action in 8 young and 10 elderly non diabetic men. Participants were studied as outpatients whilst on their usual diet (young: C=41%, F=42%, elderly: C=49%, F=37%) and following 3-5 days on liquid formula experimental diets (C=85%, F=0). This very high carbohydrate diet had no significant effect on insulin action in either the young or elderly. Following a low-carbohydrate diet (C=30%, F=55%) in the young subjects, insulin sensitivity was decreased compared to both the base-line and high carbohydrate diets. However differences based on the effects of liquid formula diets cannot be automatically extrapolated to habitual diets.

Hoffman et al. (1982) studied 7 obese NIDDM patients. Following 3 days on a base line diet (C=42%, F=35%, fibre=28 g), subjects consumed a high carbohydrate, high-fibre diet (C=68%, F=11%, fibre=81 g) for 10 days in metabolic ward. Insulin sensitivity, as assessed by the hyperinsulinemic euglycemic clamp, did not change.

Riccardi and Rivellese (1991) found that insulin-stimulated glucose disposal was significantly increased after a high-monounsaturated fat diet compared with the high-carbohydrate-low fat diet in nine NIDDM patients after 15 days.

Ciardullo et al. (1993) noted a significant increase in insulin sensitivity in NIDDM patients who consumed a high monounsaturated fat diet compared with those on a high carbohydrate-low fat diet after 15 days.

Parillo et al. (1990) compared diets containing either 40% or 60% of energy as carbohydrate (containing 40% or 20% fat, respectively, and well matched for protein, fiber, saturated fat and monosaccharides) over 15 days in 9 NIDDM subjects. This was a cross-over design carried out in a metabolic ward. Insulin-mediated glucose disposal, assessed using the euglycemic clamp at an insulin level of approximately 80 mU/l was actually significantly diminished following the high carbohydrate diet.

In addition, direct measurements of insulin sensitivity by the euglycemic hyperinsulinemic glucose clamp study showed that in comparison to high monounsaturated fat diets, high carbohydrate diets caused either no change or a decrease in insulin sensitivity in NIDDM patients (Garg et al., 1992; Parillo et al., 1992).

In summary, efforts to reduce insulin resistance by nonpharmacological means (diet and exercise) are a crucial component of the management of NIDDM (Consensus Statement, 1995). It seems that a combination of both environmental and genetic factors could influence the overall insulin resistance state (Walker and Alberti, 1993). However, there are only few studies which have directly assessed the effects of dietary carbohydrate / fat changes on *in vivo* insulin action in man. Overall they do not warrant the drawing of any strong conclusions (Storlien et al., 1991a).

2.2.5 The effect of medication on insulin resistance:

Several types of anti-hypertensive drug are known to affect insulin sensitivity and insulin secretion. Thiazide diuretics, B-adrenergic blocking agents, and calcium blockers have been shown to decrease insulin secretion (Lithell, 1991; Kawamori et al., 1993).

Angiotensin converting enzyme inhibitors, and α-adrenergic blocking agents captopril, enalapril, and prazosin have been shown to increase insulin sensitivity (Harper, 1991; Kawamori et al. 1993; Vuorinen-Markkola and Yki-Jarvinen, 1995).

2.2.6 The effect of alcohol consumption on insulin sensitivity:

Light to moderate alcohol intake is correlated with increased insulin-mediated glucose uptake, lower plasma glucose and insulin levels in response to oral glucose, and a higher HDL-C level. The changes in insulin and glucose metabolism may contribute to the lower risk of coronary heart disease described in light to moderate drinkers (Facchini et al. 1994; Simopoulos, 1994a; Simopoulos, 1994b).

Razay et al. (1992) have reported that women drinking a moderate amount of alcohol had lower plasma insulin concentrations and higher HDL-C levels than non-drinking women.

2.2.7 The effect of smoking on insulin sensitivity:

Frati et al. (1996) has reported that cigarette smoking acutely worsens glucose tolerance in healthy non-smokers as well as in usual tobacco smokers. In chronic smokers this impairment was more major than in non-smokers. This effect is probably due to diminished sensitivity to insulin since the insulin sensitivity index decreased significantly after smoking compared with the test without smoking.

2.2.8 Measurement of insulin sensitivity in man:

There are numerous methods of measuring insulin action *in vivo*. In Table 2.1 some of the advantages and disadvantages of each method are shown. Most methods can be grouped into one of three major categories.

In the first one the changes in glucose and/or insulin concentration are measured after administration of glucose (either orally or by injection) and/or insulin. For example if the insulin concentrations result are greater than those observed in a group of control subjects then insulin resistance is presumed to be present. Therefore, while this method is simple, but it only shows an indirect measurement of insulin action.

The second category of tests is the combination of glucose and insulin concentration in fasting stage or following injection or infusion of glucose with a physiological model. This model is used to derive indices of insulin action and secretion. But somehow the value of the indices depends not only on the adequacy of the model, it also depends on the ability of the model to accurately identify the required rate constants. This could be particularly problematic when insulin secretion is impaired or when glucose concentrations are rapidly changing prior to commencement of the test. Different modifications have been introduces, including injection of tolbutamide or insulin at the same time as glucose, assuming that peripheral injection of insulin has the same biological effect as does secretion of insulin into the portal venous system.

Despite this theoretical concern that most models are only able to provide a composite measure of insulin's effects on hepatic and extrahepatic tissue, some investigators have noticed that both the 'minimal' model and the CIGMA' appear to provide reproducible measures of insulin action that correlate with those obtained with other methods.

The third category includes the so-called insulin suppression tests and the variants of the glucose clamp technique approaches seek to compare insulin action in the presence of the same insulin concentration. While the former method permits

glucose concentration to change (i.e. the higher the glucose concentration the more 'insulin resistance' the subject), the latter generally keeps glucose concentrations constant and equivalent in all subjects (Alzaid and Rizza, 1993).

Of these methods, the euglycemic glucose clamp technique, although it is time-consuming and requires sophisticated equipment and highly trained personnel, is considered the "gold standard" against which all other techniques are measured (Bergman et al., 1987). By maintaining glucose constant and equal in all groups this method avoids hypoglycaemia or hyperglycaemia.

The glucose infusion rate required to maintain euglycemia at a given insulin concentration equals the sum of the insulin-induced decrease in glucose production and stimulation of glucose uptake. Since the liver takes up very little glucose in the present of euglycemia, an increase in glucose uptake is primarily due to enhanced disposal by extrahepatic tissue (Alzaid and Rizza, 1993).

Table 2.1 Methods of assessment of insulin sensitivity in man (Alzaid and Rizza, 1993).

Method	Description	Comments
Oral glucose tolerance test (OGTT)	Plasma glucose (G) and insulin (I) are measured following a standard glucose load. G/I ratio is calculated. The higher the ratio the greater the insulin resistance.	Poorly reproducible, variation in glucose absorption, G/I ratio dependent on insulin clearance as well as secretion. May provide some general information on the overall body sensitivity to insulin.
OGGT with insulin injection	Glucose load and intravenous (i.v) bolus of insulin given simultaneously. Plasma glucose is measured over 60 min. and integrated response is calculated.	Suffers from same limitations as OGGT; may result in changes in counter-regulatory hormones.
Insulin tolerance test (ITT)	A bolus of insulin (≈0.1 unit/kg) is given i.v. and the rate of decline of plasma glucose is determined (the faster the fall, the more sensitive the person).	Potential risk of hypoglycaemia; attendant counter-regulatory hormonal response may confound interpretation. In 'safe hands' and with the right dose can provide index of insulin activity.
Homeostasis model assessment (HOMA)	Only basal (fasting) plasma glucose and insulin are required.	Simple, cheap and no artificial stimulus involved. Dependent on precision of insulin assay and assumptions of model.
Continuous infusion of glucose with model assessment (CIGMA)	Plasma glucose and insulin concentrations are measured at the end of a 60 min. constant glucose infusion (5 mg/min./kg body weight). Values incorporated into a model for index of insulin action. Gives index insulin secretion.	Excellent correlation with the clamp method. can be confounded by the presence of glycosuria. Dependent on precision of insulin assay and validity of model assumptions.
Minimal model	Plasma glucose and insulin are frequently sampled over 180 min. following i.v. glucose bolus. simulation is used to analyse glucose and insulin dynamics. Gives an index of insulin action and secretion. In its modified form, an i.v. bolus of tolburamide or insulin is given at 20 min. to increase insulin concentrations.	Simple, minimally affected by ambient glucose, allows simultaneous assessment of the 5-cell function and the effectiveness of hyperglycaemia. Dependent on validity of model assumption.
Insulin suppression test (IST)	Endogenous insulin secretion is suppressed with somatostatin coupled with a fixed constant glucose and insulin infusion. Steady-state plasma glucose (≈90 min.) represents index (the higher the glucose, the greater the magnitude of insulin resistance)	Highly correlated with the clamp method. Measures insulin action at comparable insulinemia; makes assumptions about effects of drugs or hormone on insulin action. Measures composite effects of hyperglycaemia and hyperinsulinemia.
Glucose clamp: euglycemic, hyperglycaemic	Insulin (40 mU/m^2 /min.) is infused systematically and plasma glucose is maintained constant by exogenous glucose infusion. Glucose is 'clamped' at either ambient glucose concentration (euglycemia) or above ambient level (hyperglycaemia). Glucose infusion rate equals sum of decrease in hepatic glucose release and increase in glucose uptake.	quantifies (ie. gives 'real numbers') to insulin action. can be used to generate dose-response curves. Can be used to determine the site of defect of insulin action (ie. hepatic vs. extrahepatic). Depends on the skills of investigator.

2.3 Characteristic of NIDDM:

2.3.1 Introduction:

Diabetes mellitus is a metabolic disorder characterized by hyperglycaemia and alterations in fat and protein metabolism and by the occurrence of a specific set of long-term microvascular and neurologic complications (Nathan, 1996). The DCCT trial, in which improvements in diabetic hyperglycaemia in patients with insulin-dependent diabetes mellitus were shown to result in fewer microvascular and neurological complications, is the clearest demonstration of the causal link between hyperglycaemia and complications. In all probability the casual relationship will be equally clear in NIDDM when current studies are complete (see discussion by Nathan, 1995); for practical purposes the association is assumed in the management of NIDDM.

In all patients and ethnic groups, most patients with NIDDM have similar characteristics, including both insulin resistance and ß-cell dysfunction, which appear to be the basic metabolic abnormalities leading to the disease (Saad et al., 1992; Lillioja et al., 1993; Knowler et al., 1995).

2.3.2 Metabolic disorders in NIDDM:

Individuals with NIDDM characteristically display **hyperglycaemia** (Zeman, 1991). Although it seems clear that hyperglycemia must be important, it has been quite difficult to prove a relationship between hyperglycemia and diabetic complications (Raskin, 1994; Vaccaro et al., 1996). However there are data which suggest that hyperglycaemia per se might induce hypertension (Ceriello et al., 1993).

One of the other characteristics of NIDDM is increases in **glycosylated hemoglobin, (Hb)A1c**. Hemoglobin can combine non-enzymatically with glucose, and the haemoglobin conjugate, hemoglobin A1c, can be measured in blood. It increases in concentration roughly in proportion to the degree of sustained hyperglycaemia and is used clinically as a tool to indicate the degree of control of hyperglycaemia in people with diabetes (Steinberg, 1990).

Giugliano et al. (1992) has reported a significant correlation between HbA1c and classic risk factors for cardiovascular disease such as hypertension and cholesterol in diabetic patients. Nevertheless there is evidence that the risk of cardiovascular disease is not directly related to HbA1c or diabetes control and in the Whitehall study in people with impaired glucose tolerance the risk of cardiovascular disease equals that of diabetes (Reaven, 1996)

Another major characteristic of NIDDM is abnormalities in **insulin secretion or concentration**. Since the discovery of insulin 70 years ago, its effect on carbohydrate, fat and protein metabolism has been well established (DeFronzo et al., 1992). Insulin is secreted by the pancreas and acts in two major ways: (1) it has a key role in the metabolism of carbohydrate, lipid, and proteins, and (2) it has growth-promoting effects on DNA synthesis, cell division, and cell differentiation (Vander et al., 1994).

The San Antonio Heart Study showed that in Mexican Americans and non-Hispanic whites, elevations of plasma insulin concentration precede the development of hypertension, decreased HDL cholesterol, increased triglyceride concentrations and NIDDM (Nosadini et al., 1993). A causal role for hyperinsulinaemia in the development of hypertension has not been proved (Vaccaro et al., 1996), but several authors have proposed hyperinsulinaemia as the link between

hyperglycaemia and hypertension (Modan et al., 1985; Cambien et al., 1987; Reaven, 1988; Saad et al., 1990).

C-peptide is a biologically inactive peptide which is cleaned from proinsulin, the precursor of insulin, during insulin production. The flux of C-peptide into the circulation is a measure of the flux of insulin.

Taylor and Agius (1988) have proposed **C-peptide** concentration is normal in NIDDM. On the contrary, other studies have reported that elevated C-peptide concentration has been associated with increased risk of development of NIDDM (Saad et al., 1989; Haffner et al., 1990; Bergstrom et al., 1990; Charles et al., 1991).

Bergstrom et al. (1990), in a longitudinal prospective study on NIDDM patients, have reported that an increased fasting C-peptide level, which reflects insulin resistance, and increased deposition of intra-abdominal fat appear to be antecedent events in the pathogenesis of NIDDM.

2.3.3 Lipid and lipoprotein abnormalities in NIDDM:

Dyslipidaemia is frequently found in diabetic patients (Reaven, 1988). The characteristic lipid profile of individuals with NIDDM consists of elevated total and very low density lipoprotein (VLDL) triglycerides, with depleted high density lipoprotein (HDL) cholesterol levels (Howard, 1994).

The Prospective Cardiovascular Münster (PROCAM) study noted that a rise in serum triglycerides and a reduction in HDL cholesterol were clearly apparent in the

diabetic population compared with non-diabetic people (Assmann and Schulte, 1988).

Stern et al. (1989) found that more than 40% of NIDDM individuals in the San Antonio Heart Study were hyperlipidaemic, according to the criteria of the National Cholesterol Education Program; a further 23% had hypertriglyceridaemia and/or low levels of HDL cholesterol. Therefore, more than 60% of NIDDM subjects in this population had some degree of dyslipidaemia. In contrast, less than 25% of the non-diabetic subjects were hyperlipidaemic.

Several studies indicate that lipid and lipoprotein alterations found in long-term diabetes mellitus contribute to the development of late macrovascular atherosclerotic complications (Assmann et al., 1988; Stern et al., 1989; Yoon et al., 1993; Csazar et al., 1993).

Primary and secondary hypertriglyceridemias are the most common lipid transport disorders found in patients with obesity and diabetes mellitus (Eisenberg, 1987).

In summary, some of the lipid abnormalities in NIDDM patients are:

a) **Increased triglyceride production** (Howard, 1987; Durrington, 1995; Bhatnagar et al., 1996; Stern, 1996)

b) **Decreased lipoprotein lipase activity** (Howard and Howard, 1993; Durrington, 1995);

c) **Increased VLDL, especially VLDL-TG** (Kostner and Karadi, 1988; Howard and Howard, 1993; Nettleton, 1995);

d) **Increased IDL and remnant formation** (Malasanos and Stacpoole, 1991; Kasama et al., 1987);

e) **Increased cholesterol synthesis** (Duell and Bierman, 1990; Malasanos and Stacpoole, 1991);

f) **Increased cholesteryl ester transport protein** (Malasanos and Stacpoole, 1991);

g) **Increased LDL cholesterol** (Harris, 1991);

h) **Decreased HDL-C** (Kostner and Karadi, 1988; ((Malasanos and Stacpoole 1991; Stern and Haffner, 1991; Durrington, 1995; Stern, 1996)

HDL appears to protect against the development of premature coronary atherosclerosis, probably through the reverse cholesterol transport process (Miller and Miller, 1975). The Framingham Study has provided evidence that plasma HDL-C concentration is inversely related to the development of a myocardial infarction in both men and women (Miller and LaRosa, 1991). Elevated HDL levels have been shown to lead to regression of atherosclerosis in animal models (Johnson et al., 1991).

Based on the data from human and animal models in which VLDL remnants accumulate, IDL particles can be assumed to be atherogenic (Sehayek and Eisenberg, 1994), and the concentration of IDL is increased in NIDDM (Kasama et al., 1987). Epidemiological studies in humans suggest that plasma concentration of IDL or remnant lipoproteins are predictors of the severity or progression of atherosclerosis (Lewis and Steiner, 1996). Nordestgaard et al. (1992) have suggested that IDL or remnant lipoprotein concentrations are better predictors of the extent of atherosclerosis than were LDL or VLDL. Cholesterol-fed animals also demonstrate accretion of IDL and rapid development of atherosclerosis (Nordestgaard and Lewis, 1991).

Csazar et al. (1993) in their study of two large ethnically different populations of diabetic patients found no evidence of a contribution of Lp(a) to the increased risk for atherosclerosis in diabetes. They concluded that mechanisms independent of genetic apo(a) types and Lp(a) levels are responsible for the development of diabetic vascular complications (Heesen et al., 1993; Csazar et al., 1993).

Apo(a) seems to be little affected by age, sex, and diet (Nestel et al, 1993), although a recent study has reported that the apo(a) level was increased 25% by the trans fatty acid, elaidic acid in the diet (Nestel et al., 1993). Haffner et al. (1992) reported that Lp(a) levels were significantly higher in diabetic patients who had higher total and LDL cholesterol levels.

Billingham et al. (1989) reported an elevation of apo B levels in newly diagnosed NIDDM patients as well as in patients treated by diet alone or by diet and glibenclamide. Also the concept that overproduction of VLDL apo B is one of the causes for the elevation of VLDL in NIDDM has been proposed (Taskinen, 1990).

A diet high in cholesterol content increases the plasma CETP concentration in man (Martin et al., 1991). Marcel et al. (1990) reported that the levels of CETP are 25% higher in women compared with men. Also it has been reported that the expression of CETP in transgenic mice is associated with increased susceptibility to atherosclerosis (Maroti, 1993).

Consistent changes in LDL concentration are not always observed in individuals with NIDDM (Howard et al., 1984; Stern et al., 1984; Howard and Howard, 1993). It is possible that abnormalities in flux through the LDL compartment, as

well as abnormalities in LDL composition may be very relevant to understanding atherosclerosis (Hoard and Howard, 1993).

2.3.4 Non-Esterified-Fatty Acid (NEFA) and NIDDM:

It has been shown that ambient plasma NEFA concentrations are higher than normal in NIDDM patients (Park, 1993; Reaven, 1995; Durrington, 1995). The elevation in VLDL and triglycerides has been linked to insulin resistance via elevated circulating NEFA levels (Howard, 1994).

It is proposed that the increase in plasma NEFA concentration that occurs when insulin-resistant individuals can not maintain a state of compensatory hyperinsulinaemia is primarily responsible for the development of significant hyperglycaemia in patients with NIDDM. As the plasma NEFA and glucose concentrations increase, beta-cell secretory function will be further compromised (Reaven, 1995).

Paolisso et al. (1995) have reported that high fasting plasma NEFA concentration is a risk factor for NIDDM, independent of sex, percent body fat, insulin-mediated glucose uptake and fasting TG concentration. Walker (1995) have noted that only obese NIDDM patients have high plasma NEFA concentration.

2.3.5 Cholesteryl ester transfer protein (CETP) abnormality and NIDDM:

Cholesteryl ester transfer among plasma lipoproteins is a critical step in the formation and metabolism of cholesterol esters and of lipoproteins (Tomkin and Owens, 1994). Cholesterol becomes esterified by the enzyme lecithin cholesterol acyltransferase (LCAT) forming cholesteryl ester mainly in the HDL fraction. Interchange of core lipids (cholesteryl esters and triglyceride) between all

lipoprotein classes is mediated by cholesteryl ester transfer protein. As a result mass transfers of cholesteryl ester from HDL to LDL, IDL, and VLDL occur, and HDL becomes preferentially enriched in triglyceride. HDL triglyceride is hydrolysed by hepatic triglyceride lipase; resulting in small HDL particles. LDL particles also become smaller and more dense by a similar process. The resulting lipid profile is characterised by low HDL cholesterol, small LDL particles, and is frequently found in the plasma of patients with NIDDM. An increase in CETP activity in NIDDM, together with increased plasma triglyceride, may by determining this potentially atherogenic dyslipidemia, increase the cardiovascular risk in NIDDM patients (Laakso et al., 1993).

2.3.6 Obesity, body fat distribution and NIDDM:

It has been a frequent finding that Type II diabetes is associated with obesity. Approximately 80% of patients with NIDDM are obese (Chung et al., 1995).

The mechanism underlying this association are still poorly understood. However, recent studies suggest that insulin resistance may play a key role in the pathophysiology of the metabolic abnormalities associated with obesity (Skarfors et al., 1991; Daly and Landsberg, 1991), particularly abdominal obesity (Fontbonne et al., 1992; Stern, 1996).

There is strong evidence that the pattern of distribution of body fat is more strongly related to NIDDM than are simple measurements of body fatness (Hartz et al., 1983; Shuman et al., 1986). The excessive adipose tissue in obesity tends to be distributed in the upper body, including the waist (android or upper body obesity) or the lower body, including the hips and buttocks (gynoid or lower body obesity). The former is more characteristic of men and is more closely associated with Type II diabetes, hyperlipidaemia and other metabolic disorders while the latter is more

usually seen in women and has fewer adverse metabolic implications (Gurr & Harwood, 1991).

Eighty percent of NIDDM patients exhibit truncal obesity (Harper, 1991). More specifically, visceral fat is important in relation to cardiovascular risk factors, since visceral fat is closely associated with the presence of dyslipidemia. Studies in both men and women have demonstrated that the accumulation of visceral fat is linked to high plasma triglyceride levels and reduced HDL cholesterol concentration. Furthermore, visceral adipose tissue is also associated with insulin resistance, hyperinsulinemia and glucose intolerance (Van Gaal et al., 1995).

While visceral fat is difficult to measure (multiple MRI measurements would be a "gold standard"), surrogate measures may be used. Para-vertebral intra-abdominal fat may be estimated by a combination of dexal energy x-ray absorbtiometry and measurement of skin-fold thickness (subcutaneous fat). Most epidemiological studies on the subject use the ratio of waist to hip circumference as an indicator of intra-abdominal or visceral fat.

Hodge et al. (1993) have studied the contribution of body fat distribution to coronary vascular disease risk in Nauruans, a population known for its very high prevalence of obesity and NIDDM. They reported independent and significant associations between the waist:hip ratio, body mass index, and coronary vascular disease risk.

2.3.7 Family History of Diabetes Mellitus:

Several studies on populations at high risk for developing NIDDM have showed that history of diabetes in a first degree relative is a powerful predictive factor for the development of diabetes and impaired glucose tolerance preceding NIDDM is

more prevalent in non-diabetic offspring of diabetic patients than in control subjects without a positive family history of diabetes (Leslie et al., 1986, Haffner et al., 1988; Eriksson et al., 1989).

Risk of developing diabetes increases with the extent of diabetes in previous generations and offspring of diabetic parents develop diabetes at a younger age than their parents (Viswanathan et al., 1985; Ramachanran et al., 1988).

Several studies conducted in Europid populations have suggested a substantial excess maternal transmission in NIDDM (Kobberling and Tillil, 1982; Alcolado and Alcolado, 1991; Thomas et al., 1994), although some studies have found an excess of paternal transmission for NIDDM patients (Young et al., 1994; Mitchell et al., 1995). The influence of maternal or paternal transmission of a tendency to NIDDM might well differ in different populations (Viswanathan et al., 1996).

2.4 Dietary management in NIDDM:

2.4.1 Introduction:

Although dietary treatment is accepted as an integral part of the management of NIDDM (Milne et al., 1994), opinion regarding an optimum dietary regimen for individuals with NIDDM remains unsettled, even though no other disease in medical history has been treated by dietary means more intensively (Marmot, 1992). The aim of dietary intervention in NIDDM is to achieve normal blood glucose control and optimal serum lipid levels, while preventing, or delaying the progress of, long term complications associated with the condition (American Diabetes Association, 1994). Chronic complications of NIDDM include cardiovascular disease, nephropathy, neuropathy, and retinopathy (Zeman, 1991). Both the chronic complications and dyslipidaemia associated with NIDDM have been investigated in relation to an optimal dietary regimen for individuals with NIDDM.

A dietary approach for patients with NIDDM should also aim to decrease very-low-density lipoprotein (VLDL) triglyceride and increase plasma high-density lipoprotein (HDL), as these two factors influence the risk of macrovascular disease (Reaven, 1988a).

2.4.2 Present dietary recommendations for NIDDM:

The dietary regimen currently recommended for people with NIDDM is similar to those of the National Heart Foundation and the 1993 Dietary guidelines for Australians (National Health and Medical Research Council 1992). The recommendations include consuming a diet high in complex carbohydrates (≥50% of total energy intake), and low in fat (<30% of total energy intake) (Dietitians Association of Australia, 1990). Less than 10% of calories should come from saturated fat, and no more than 10% from polyunsaturated fat, thus monounsaturated fats make up the balance. Sucrose or other "simple sugars" need not to be avoided, but rather are incorporated into the total carbohydrate content for the diet. The recommended dietary fiber intake per day is 20 to 35 g. In regard to protein intake, insufficient evidence exists to support protein intake lower than 10% or higher than 20%, for the general population (Coulston, 1994a).

Also the American Diabetes Association (ADA) recommends that dietary intake of fat is reduced to no more than 30% of total calories (ADA Consensus Statement, 1993). Although the European Association for the Study of Diabetes advises a similar amount of total fat, it suggests that total fat intake can be higher provided that only monounsaturated fat is increased and not saturated fatty acids (Riccardi and Rivellese, 1991).

Even though the primary purpose of this dietary recommendation is to reduce the risk for coronary heart disease (Grundy, 1991) it has been found to aggravate the dyslipidaemia associated with NIDDM (Spiller, 1991), and hence increase the risk of developing chronic complications. This is due to the fact that a high carbohydrate, low fat diet has been found to increase serum triglyceride levels (Grundy, 1991), and decrease plasma high density lipoprotein (HDL) levels (Coulston et al., 1989).

2.4.3 Carbohydrate consumption and NIDDM:

Carbohydrates are classified into three major types: monosaccharides; disaccharides; and polysaccharides. Monosaccharides are the simplest form of carbohydrate and are referred to as simple sugars. Disaccharides are referred to as double sugars as they are composed of two sugar units. The polysaccharides contain many sugar units. The monosaccharides and disaccharides are referred to as sugar or simple carbohydrates; polysaccharides are called starches or complex carbohydrates because they have a more intricate chemical structure (Eschelman 1991). Once eaten, all carbohydrates are digested to glucose. This process occurs at different rates depending on the type of carbohydrate and whether or not it occurs in a food with dietary fibre. The glucose is then available for use as energy by the cells.

However, it is generally recommended that people produce their body's glucose from complex carbohydrate foods or from the sugars in fruit (fructose) or milk (lactose) rather than from straight sugar (glucose) because these foods also supply other important nutrients such as vitamins and fibre in fruit. In addition, there is also a difference in the rate at which glucose arrives in the blood from different foods. After eating sugar, blood glucose levels tend to rise fairly quickly. This in turn causes the body to produce more insulin which can hinder the body's ability to use fats as an energy source (Stanton, 1992).

The glycaemic index (GI: ranking of foods based on their immediate effect on blood sugar levels) allows one to divide foods into those with a low, medium and high glycaemic index. Sugar does not have a higher glycaemic index than some complex carbohydrates - depending on the physical properties and preparation of the carbohydrate. The belief that a diet weighted in favour of "low glycaemic index" foods will advantage a person with diabetes (by lowering the blood glucose) is attractive, with some experimental evidence to suggest it. However, a diet comparison of dietary with a fat-modified diet has not been done, and the place of a low GI diet in clinical practice is, at present, uncertain.

2.4.3.1 Dietary carbohydrate and blood lipids and lipoproteins:

A high carbohydrate diet increases plasma TG concentration but not that of cholesterol. The effects of dietary carbohydrates depend on the type of carbohydrate. Intake of sucrose or fructose within the range usually found in the population does not have any elevating effect on plasma triglycerides in most normal and diabetic subjects (Truswell, 1994). While the plasma TG levels increase, HDL-C levels decrease (Gonen et al., 1981, Brinton et al., 1990).

Although high carbohydrate low-fat diets can decrease LDL cholesterol, they are also associated with other metabolic alterations in diabetes patients. Those who have elevated levels of LDL-C would benefit from a decrease in plasma cholesterol; however, the dyslipidemia associated with diabetes most commonly consists of an elevation of plasma triglyceride and decreased HDL-C concentration. These lipid abnormalities have been shown to be associated with an increased risk for CVD, especially in NIDDM patients (Coulston et al., 1987; Garg et al., 1988; Coulston, 1994).

Therefore, a low-fat high carbohydrate diet in patients with NIDDM leads to 1) higher day-long plasma glucose, insulin, and TG concentrations; 2) post-prandial accumulation of TG-rich lipoproteins of intestinal origin; 3) decreased production of VLDL-TG; and 4) increased post heparin lipoprotein lipase activity (Chen et al., 1995).

2.4.4 Fat consumption and NIDDM:

The use of fat as a replacement source of energy for some of the carbohydrate has been suggested (Garg et al., 1988; Marmot, 1992). Both saturated fat and polyunsaturated fat have been associated with factors which can increase the risk of developing cardiovascular disease (Marmot, 1992). However, this adverse effect may be avoided by the selection of unsaturated fatty acids like monounsaturated fatty acids which has shown to lower levels of LDL cholesterol effectively without reducing levels of HDL cholesterol (Garg et al., 1988; Garg et al., 1992; Campbell et al., 1994; Coulston, 1994).

2.4.4.1 Saturated fat consumption:

These fats tend to dominate the fatty acids present in fatty meats, some dairy products, palm oil, coconut oil, butter, cream, chocolate and most fast foods. Saturated fatty acids are a special risk factor for heart disease since their consumption increases the undesirable LDL cholesterol and decreases the protective HDL cholesterol; they may also decrease insulin sensitivity (Simopoulos, 1994).

Animal studies have suggested that a diet rich in cholesterol and saturated fat (an atherogenic diet) enhances the plasma CETP activity (Stein et al., 1990, Quinet et al., 1990). Increased CETP activity may result in decreased levels of HDL-C and increased levels of VLDL-C and LDL-C (Grundy and Denke, 1990).

Therefore, the major nutritional recommendations for NIDDM include reducing saturated fat and cholesterol in the diet ; with the aim of reducing the high incidence and prevalence of cardiovascular disease in NIDDM (ADA, 1993).

2.4.4.2 Polyunsaturated fat consumption:

Polyunsaturated fatty acids tend to dominate the fatty acids present in vegetable oils and polyunsaturated margarines. Many nuts and seeds are also rich in polyunsaturates.

The consumption of polyunsaturated fatty acids can lower cholesterol levels and may have other potential benefits for NIDDM, but there is concern over using these compounds in large amounts and for prolonged periods. No population has ever consumed these compounds in large amounts over extended periods of time. Potential adverse effects, such as lowering HDL-C especially when consumed in large quantities, have been reported with polyunsaturates (Grundy and Denke, 1990; Grundy, 1991).

Long-chain ω-3 fatty acids, found predominantly in fish oil, have a hypotriglyceridemic action, and fish oil supplements have been shown to reduce serum TG level in NIDDM patients (Friday et al., 1989; Hendra et al., 1990; Kasim, 1993). However fish oil has also been shown to have adverse effects on glycemic control in NIDDM (Glauber et al., 1988; Kasim et al., 1988; Friday et al., 1989; Borkman et al., 1989; Vessby 1989; Stacpole et al., 1989) and a tendency to elevate LDL-C (Jenkins et al., 1989; Kasim, 1989; Malasanos and Stacpoole, 1991; Connor et al., 1993).

In another study, glucose was not affected by a dose of 4 g of ω-3 FA per day but was increased at a dose of 7.5 g per day (Schectman, Kaul, and Kissebah 1988). In still another study, Hendra et al. (1990) reported that glucose was increased after 3 weeks but not after 6 weeks of consuming 3 g of ω-3 FA per day, suggesting that short-term studies may be of limited value. Eight other short-term trials have failed to demonstrate a change in glucose tolerance (Popp-Snijders et al., 1987).

Following the report of adverse effects of rather high levels of fish oil in the diets in elevating plasma TG in streptozotocin (STZ) diabetic rats, (primarily a model for IDDM) (Illman et al., 1986; Popp-Snijders et al., 1986) drew attention to their findings with ω-3 FA in six NIDDM subjects. When fed a daily supplement of 3 g of ω-3 FA for 8 weeks, all NIDDM subjects showed a decrease in plasma TG. These authors emphasized the observation that relatively small amounts of ω-3 FA can bring about improvements in plasma TG without diminishing glucose control (Popp-Snijders et al., 1987; Popp-Snijders et al., 1990). Controversy about the possible adverse effects of fish oils on glucose and insulin metabolism has continued ever since (Nettleton, 1995).

Lack of information on the long-term effects of polyunsaturated fatty acid-rich diets has led to current recommendations that their intake be restricted to less than 10% of the total energy intake (American Diabetes Association, 1987; Coulston, 1994).

2.4.4.3 Monounsaturated fat consumption:

Monounsaturated fatty acids have been used in large amounts in Mediterranean countries for centuries and appear to be safe. Common oils such as olive, canola, and peanut which are high in monounsaturated fatty acids may be added to the diet in a palatable manner (Griver and Henry, 1994).

These fatty acids tend to dominate the fatty acids present in olives and olive oil, avocados and nuts, such as almond, macadamia, etc. They also occur in chicken and some fish. Meat products may contain up to 50% of total fats as monounsaturated fatty acids, but are also rich in saturated fats and cholesterol and thus should not be considered good sources (Garg, 1994).

Recent research has shown that monounsaturated fatty acids are more beneficial than previously thought because they reduce the undesirable LDL cholesterol and raise the protective HDL cholesterol.

Oleic acid, or ω-9 fatty acid has attracted attention for its possible hypotriglyceridemic effect. Its beneficial effect on serum TG, HDL, and its lack of negative effect in increasing LDL-C commend further studies in hyperlipidemia (Jenkins et al., 1989).

Because polyunsaturated fatty acids have the potential to lower HDL cholesterol and apo AI levels, cis-monounsaturated fatty acids seem to be the best choice for replacement of dietary carbohydrates in diabetic patients (Garg, 1994; Campbell et al., 1994).

From the point of view of CHD the Mediterranean diet (high in oleic acid, ω-9 fatty acid) is not only non-toxic in having a limited amount of dietary saturated fatty acids and cholesterol but also protective in providing an abundant supply of ß-carotene as well as vitamins E and C. If these explanations are upheld, then they offer a variety of options for modifying the risks to health and emphasize the benefits of the Mediterranean diet (James et al., 1989).

2.4.4.4 Dietary cholesterol and blood lipids and lipoproteins:

Fungwe et al. (1992) have reported that increasing dietary cholesterol intake increases the concentrations of TG and cholesteryl ester in liver and plasma in rat. This is accompanied by reduced fatty acid oxidation and increased incorporation of exogenous fatty acid into hepatic TG and increased secretion of VLDL (Fungwe et al., 1992).

Interestingly, Fungwe et al. (1994) have recently suggested that dietary cholesterol stimulates the biosynthesis of FFA, while the addition of TG to the diet stimulates production of cholesterol.

Dietary cholesterol intake causes elevation in the plasma total cholesterol and LDL-C (Erickson et al., 1964; Hegsted et al., 1965). Studies have shown that the serum cholesterol response to dietary cholesterol tends to plateau at high cholesterol intakes (Keys, 1984; Hegsted, 1986). Riccardi et al. (1987) suggested that the most effective dietary change to reduce plasma cholesterol concentrations in human is to decrease the consumption of saturated fat and cholesterol.

2.4.5 Fibre consumption:

Dietary fiber is generally categorized according to its water solubility into the insoluble non-carbohydrate forms such as cellulose and lignin and the soluble pectines, gums and mucilage. Recommendations about dietary fiber generally refer to the total of insoluble and soluble types (Griver and Henry, 1994).

Hydrosoluble fibre (guar, pectines) has been shown to improve carbohydrate and lipid metabolism, possibly by decreasing the intestinal absorption of glucose, fatty

acids and cholesterol (Pagnan and Bonanome, 1993). However, Riccardi and Rivellese (1991) noted a significant reduction in HDL-C after subjects with NIDDM had consumed a fibre-rich high carbohydrate diet. The reason for the reduction in HDL-C is uncertain.

In general, evidence that fibre in fruits and vegetables has some cholesterol - lowering effect is better than for most of the cereals so far studied. One aim of a lipid-lowering diet must be to decrease saturated fat consumption and increase fibre intake (Durrington, 1995). Therefore, in practice, the consumption of legumes, vegetables, and fruit should be encouraged (Riccardi and Rivellese, 1991) and an intake of up to 40 g fibre/day or 25 g/1000 kcal of food intake appears to be beneficial (Position statement, 1993).

Nuttal (1993) believes it is not possible to ascertain the effect of an increase in fibre consumed in naturally occurring foods. He suggests that this is due to the fact that; (1) usually when the carbohydrate content of the diet is increased then the fat content for the diet is decreased, (2) the sources and types of carbohydrate change, and (3) the digestibility of various starches differ from one another. Soluble fibre has however been shown to have a significant effect on the serum total and LDL cholesterol concentrations in individuals with NIDDM (Nuttal, 1993).

Overall, Nuttal (1993) concluded that even if a large amount of dietary fibre was consumed, its use in the management of blood glucose concentration in individuals with NIDDM is limited. However he felt that a diet high in soluble fibre, where fruits and vegetables are emphasised, may be justifiable due to the other effects of soluble fibre. Putting the discussion on fibre and its effect on blood glucose control aside, an adequate fibre consumption is definitely important in both people with and without diabetes in order to promote gastrointestinal motility (Coulston, 1994).

The mechanisms by which dietary fibre acts as a blood glucose - lowering and lipid - lowering agent are not clear. Several mechanisms have been proposed including increasing the faecal excretion of bile acids and decreasing the rate of lipid absorption in the large intestine (Schneeman and Tietyen, 1994). The glucose - lowering effect may be through the effects of fibre on slowing gastric emptying and thus slowing the absorption of glucose.

2.4.6 Protein consumption:

Because protein may potentially exert both beneficial and adverse effects in diabetes, the optimal protein content of the diabetic diet is not currently well-defined (Griver and Henry, 1994). Protein can have a favourable effect on carbohydrate metabolism in NIDDM by stimulating insulin secretion and reducing glycemic excursions (Wylie-Rosett, 1988; Zeller, 1991). However, excess dietary protein in persons with NIDDM may lead to diabetic nephropathy by increasing the workload of the kidney (Wylie-Rosett, 1988). Therefore the recommended dietary allowance (RDA) for protein is 0.8 g/kg body weight for adults (Position Statement, 1993), or 12-20% of total calories (ADA, 1993).

2.4.7 Effects of diet on lipids and lipoproteins in individuals without NIDDM:

Ginsberg et al. (1990) in a randomized double-blinded trial involving 36 healthy young men, studied the effects of three different diets on lipid profiles. The diets were: American Heart Association (AHA) step 1 diet (C=55%, F=30%, S:M:P=10:10:10), AHA step 1 with enriched monounsaturate fat (C=47%, F=38%, S:M:P=10:18:10), and the control group (C=47%, F=38%, S:M:P=18:10:10). After 10 weeks, there was a significant reduction in plasma total cholesterol in both step 1 diets. Also LDL-C was significantly reduced in step 1 mono-enriched diet.

Mensink and Katan (1987) studied the effect of high dietary carbohydrate intake (C=62%, F=22%, M=9.3%) vs high monounsaturated fat intake (C=46%, F=41%, M=24%) on 48 healthy subjects. After 36 days of restricted diet (supplied food) they concluded that the olive-oil-rich diet, unlike the complex-carbohydrate-rich diet, caused a specific fall in non-HDL cholesterol.

In the cross-over study of 11 healthy subjects Grundy (1986) compared three different diets for 4 weeks. The high monounsaturated fat diet and high saturated fat diet contained: 40% of energy as fat, 43% as carbohydrate, and 17% as milk protein. The high carbohydrate diet had 63% of energy as carbohydrate and 20% as fat. He noted that the high monounsaturated fat diet reduced P-Cholesterol and LDL-C, but it increased the LDL:HDL-C:ratio. But the high carbohydrate diet increased P-TG and decreased HDL-C. Although the results are interesting they may not be applicable in free living conditions as all the diets were supplied in the form of liquid and participants stayed in a metabolic ward.

Griffiths et al. (1994) studied the post prandial effect of a high fat (mainly saturated) and a low fat diet on 8 normal subjects. They suggested that after ingestion of combinations of carbohydrate and fat, the action of lipoprotein lipase on chylomicron-triacylglycerol leads to direct release of fatty acids into the plasma and increased fat oxidation.

In a cross-over study of 38 healthy young adults, Wahrburg et al. (1992) compared the effect of two fat reduced diets; one enriched with monounsaturated fatty acids (C=50%, F=32%, SFA=10%, M=16%, P=4%) and the other riched in polyunsaturated fatty acids (C=50%, F=32%, SFA=10%, M=10%, P=10%). All the participants were initially on a typical western diet (C=43%, F=41%,

SFA=20%, M=13%, P=4%). After 3 weeks both diets led to significant reduction in serum Cholesterol, LDL-C, and HDL-C. The authors concluded that the diet rich in monounsaturated fatty acids may be more advantageous than that rich in polyunsaturated fatty acids because it does not lower apolipoprotein A-1 concentrations as much as the polyunsaturated -rich diet does.

Berry et al. (1992) also studied the effects of high-monounsaturated fat diet (C=49.8%, F=33.8%) and high carbohydrate diet (C=60%, F=23%) on 17 healthy males over 12 weeks. This was a cross-over study and all the food was supplied by a boarding college. They noticed a reduction in P-Cholesterol by 7.7% and LDL-C by 14.4%. No changes were noticed in HDL-C.

Abbey and Nestel (1994) examined the effect of dietary trans fatty acids on activity of CETP in plasma from 27 men in a double blind crossover comparison. The background diet, containing 15% energy as fat from dairy products, meat, bread and cereals, was supplemented with oleic or elaidic acid providing a further 20% energy. The elaidic supplement provided about 6% energy as trans fatty acid. After 3 weeks duration on each diet they found that activity of CETP in plasma was significantly higher after the elaidic acid-rich diet compared with the diet enriched with oleic acid.

In regard to the type of fat and its effect on lipids and lipoproteins Liechtenstein et al. (1993) have studied the effects of canola, corn, and olive oils on fasting and post-prandial plasma lipoproteins. There were 15 participants with LDL-C concentration >130 mg/dL who consumed 2/3 of their fat intake as one of the above mentioned oils. After being on each diet for 32 days, plasma cholesterol concentration declined; however, the declines were significantly greater for the canola (12%) and corn (13%) than for the olive (7%) oil. Mean LDL-C also was

reduced in diets containing each type of oil (16%, 17%, and 13% for canola, corn, and olive oil respectively). They concluded that although these three oils had some effects on lipid profiles, none of them had a significant advantage in altering the overall lipoprotein profile in middle-aged and elderly subjects with initially high LDL-C.

Sarkkinen et al. (1994) compared three types of dietary fat on 160 free living, moderate hypercholesterolemic subjects for six months. The diets were a control diet (F=35%, S:M:P:R=14:10:4), a AHA type diet (F=32%, S:M:P:R=10:8:8), a mono-enriched diet (F=34%, S:M:P:R=11:11:5), and a reduced fat diet (F=30%, S:M:P:R=12:8:3). LDL-C decreased significantly in the mono-enriched diet and AHA diet group . No changes were noticed in HDL-C or other lipids.

In animals, Brousseau et al. (1995) studied the effect of saturated, polyunsaturated, and monounsaturated fat diet in 10 cynomolgus monkeys. They were fed three diets that provided 30% of energy as fat with 0.1% cholesterol by weight and differed solely by the substitution of saturated, mono- and polyunsaturated fats as 60% of total fat energy. Total, LDL, and HDL cholesterol, as well as LDL apo B, HDL apo A-I and HDL total apo C concentrations, were reduced with the mono- and polyunsaturated fat diets relative to the saturated fat diet. This study concludes that dietary monounsaturated fats are comparable to polyunsaturated fats in their effects on hepatic lipid and apo mRNA levels in this species, with both unsaturated fats significantly reducing only hepatic apo C-III mRNA abundance relative to saturated fat.

2.4.8 Effects of diet on lipids and lipoproteins in NIDDM:

Ciardullo et al. (1993) studied 10 NIDDM patients in a metabolic ward, for 15 days. The patients consumed either a high monounsaturated fat diet (C=40%,

F=40%) or a high carbohydrate diet (C=60%, F=20%). They had significant reductions in fasting post-prandial blood glucose, insulin, and fasting plasma TG after consuming high monounsaturated fat diet (mainly olive oil). No significant changes were noted in LDL-C or HDL-C.

Coulston et al. (1987) studied the effect of a high carbohydrate diet (C=60%, F=20%, poly:sat:ratio=1.3) compared with a high-fat diet (C=40%, F=40%, poly:sat:ratio=1.3) on 9 NIDDM patients over 15 days. They noted that although the high carbohydrate diet contained twice as much fibre as the other diet, it increased fasting and post-prandial TG and also reduced HDL-C. No effect was seen on LDL-C.

Garg et al. (1988) in a 28 days cross-over study of 10 NIDDM patients who were receiving insulin found that the high monounsaturated fat diet (F=50%; M=33%, C=35%) improved glycemic control by reducing fasting blood sugar and insulin. Lipid abnormalities were also reduced; the high monounsaturated fat diet was associated with a reduction in VLDL-C and TC:HDL-C:ratio and an increase in HDL-C and HDL-Apo A, but no effect on LDL-C was seen [all compared to the group on the high carbohydrate diet (F=25%, C=60%)].

Abbott et al. (1989) compared the effect of a modified fat diet (F=42%; P:S:R=0.3, C=43%) vs a high carbohydrate diet (F=21%; P:S:R=1.2, C=65%) on 13 subjects (7 NIDDM and 6 control). They found that the high carbohydrate diet reduced LDL-C but had no effect on HDL-C, P.TG, or P.Cholesterol. The authors could not determine whether the reduction in LDL-C was due to an increase in vegetable and legume fiber or substitution of carbohydrate for saturated fat. The study structure is somewhat confusing as all the subjects started on a modified diet for 7-10 days and then some of them switched to high carbohydrate diet for 5 weeks.

Garg et al. (1992) in the study of 8 men with mild NIDDM noticed that in comparison to high monounsaturated fat diets, high carbohydrate diets caused a 27.5% increase in plasma TG after 21 days on the diet.

Most of the studies had looked at olive and olive oil as the sources for monounsaturated fat. Lerman-Garber et al. (1994) used avocado instead, and studied the effect of replacing saturated fat with avocado containing monounsaturated fat in 16 female NIDDM subjects. During the 4 week base-line period, all subjects received isocaloric diets. After that they were either on a high monounsaturated fat diet (C=40%, F=40%, M=24%, P:S:R=0.45), or a high carbohydrate diet (C=60%, F=20%, M=6.6%, P:S:R=1) in a crossover study design for 4 weeks on each diet, with 4 weeks wash out period between the diets. They found that both diets reduced fasting blood sugar and total cholesterol significantly. In addition, the high monounsaturated fat (avocado) diet reduced fasting TG significantly. No changes were noticed in HDL-C or LDL-C.

Rasmussen et al. (1993) compared the effect of a high carbohydrate diet (C=50%, F=10%; M=10%) to a high monounsaturated fat diet (C=30%, F=50%; M=30%) on 15 NIDDM patients. In high monounsaturated fat diet they used olive oil and some almonds (pre-packed) as the only source of monounsaturated fat. After 3 weeks the FBG, systolic and diastolic blood pressure decreased significantly in the high monounsaturated fat group. No changes was noticed in the lipid profile, perhaps because of the short duration of the study.

In another study on people with NIDDM, Campbell et al. (1994) compared the effects of a high monounsaturated fat diet (C=40%, F=38%, M=21%) to a high carbohydrate diet (C=52%, F=24%, M=8%) in 10 patients who prepared their food

at home. After 2 weeks the 24 hour urinary glucose, P-TG, and mean profile glucose levels were reduced significantly after consuming a high monounsaturated fat diet, but no effects were seen on LDL-C, HDL-C, or prandial TG.

Sheard (1995) in a cross-over study of forty-two NIDDM patients showed that compared with the high monounsaturated fat diet (F=45%; M=25%; S=10%; P=10%, C=40%, S:M:P=1), the high carbohydrate (F=30%, C=55%, P=15%) diet increased fasting plasma TG level and VLDL-C, after three weeks. No changes was noted in plasma cholesterol, HDL-C, or LDL-C.

In another study of eight NIDDM patients Rivellese et al. (1990) studied the effects of two prepared diets containing different amounts of carbohydrate (40 vs. 60%) and fat (20 vs. 40% respectively). After 15 days the high carbohydrate diet resulted in increased plasma TG, VLDL-C and VLDL-TG. No differences were noted in fasting blood sugar, LDL, or HDL level. The main source of fat in the high fat diet was olive oil.

Garg et al. (1992) in the study of 8 men with mild NIDDM noticed that in comparison to high monounsaturated fat diets, high carbohydrate diets caused 27.5% increase in VLDL-Cholesterol level, after following each diet for 21 days. No changes in plasma insulin were found.

Mensink and Katan (1992) reformed a meta-analysis of 27 trials (from 1972 to 1992) in which carbohydrate was replaced by fat in metabolic ward conditions. They found that replacement of saturated by unsaturated fatty acids raised the HDL:LDL-C:ratio, whereas replacement by carbohydrates had no effect. Replacing 10% of energy in the form of saturated fatty acids by carbohydrates lowered LDL cholesterol by 0.33 mmol/l and HDL cholesterol by 0.12 mmol/l, whereas

replacement by monounsaturated fatty acids causes a fall of 0.39 mmol/l in LDL-C and of 0.03 mmol/l in HDL-C. Use of polyunsaturated fatty acids instead of monounsaturated fatty acids would cause a slight additional fall of 0.08 mmol/l in LDL-C but also an additional decrease of 0.02 mmol/l in HDL-C. Both epidemiological and controlled clinical trials suggest that each 0.026 mmol/l (1 mg/dl) increment in LDL-C causes an increase in coronary risk of 1%. Epidemiological observations also shown an increase of 2-3% in risk for each 0.026 mmol/l decrease in HDL cholesterol (Mensink & Katan, 1992).

Blades and Garg (1995) studied the effect of a high carbohydrate diet (C=55%, F=30%), and a high monounsaturated fat diet (C=40%, F=45%) on plasma triacylglycerol in 10 NIDDM men. After 6 weeks of a cross-over study, they concluded that the high carbohydrate diet raised fasting plasma triacylglycerol concentration by 26%. No significant changes were noticed in plasma cholesterol, HDL-C, or LDL-C.

Walker et al. (1995) examined the effects of a high carbohydrate, low fat diet (C=50%, F=23%) and a modified fat diet (C=40%, F=36%) on body weight and metabolic control in 24 NIDDM patients. After 3 months on each diet in a crossover study, they concluded that although the modified fat diet is not a low-fat diet, it did not appear to facilitate weight gain in NIDDM patients living at home. There was no change in lipid profiles or HbA1c during the study.

One of the few studies which was long-term (18 months) and studied the effect of diet on NIDDM patients is the one by Milne et al. (1994). They looked at 70 patients in free living conditions and compared the effects of 3 kinds of diet: high carbohydrate, modified fat (Sat:Poly:Mono:ratio=1)diet, and weight management

diet. Despite of the continuous consultation with dietitians, no significant changes were noticed in the overall study.

As has been noted changing the amount of fat or carbohydrate can have some effects on the metabolic profiles of individuals, especially the NIDDM patients. But because of the short study periods nearly all studies have concluded that there is a need for longer studies to be able to draw definitive conclusions. In addition, most of these studies were carried out either with hospitalized subjects whose diet and lifestyle were rigidly controlled or with subjects supplied with pre-packed food, so that the effects of such diets on subjects in normal life were unclear.

2.5 Coronary heart disease and NIDDM:

Morbidity and mortality from coronary artery disease are increased in patients with non-insulin-dependent diabetes mellitus (Reaven, 1988; Ruiz et al., 1994). In fact, the incidence of heart disease among diabetic subjects is three times that of the normal population (Kannel et al., 1986).

Recently, the Multiple Risk Factor Intervention Trial (MRFIT) reported that the mortality rate for CAD and cardiovascular disease was three times higher in diabetic men than in non-diabetic men (Stamler et al., 1993). Some of these risk factors could be related to changes in lipids.

Over the last decade much attention has been focused on the close association between triglyceride and cardiovascular disease, particularly in NIDDM individuals. Results from the WHO Multinational Study indicated that ischaemic heart disease was more strongly associated with serum triglyceride than serum cholesterol levels, especially in obese NIDDM people (West et al., 1983).

Also the 11 years follow-up of patients with NIDDM in the Paris Prospective Study showed that fasting triglyceride concentration was the only factor significantly associated with mortality from coronary heart disease (Fontbonne et al., 1989).

Laakso et al. (1993) in a 7-year follow-up study discovered that both high total and VLDL triglycerides in addition to low HDL cholesterol and high VLDL cholesterol are powerful risk factors for CHD events in patients with NIDDM. The risk of CHD was doubled in those NIDDM subjects with high total triglyceride levels (>2.3 mmol/l).

Numerous epidemiological and genetic studies have shown that hyperlipoproteinemia, in particular hypercholesterolemia, is one of the principal risk factors for coronary heart disease (Douste-Blazy and Kloer, 1989; Law and Wald, 1994).

Both epidemiological and controlled clinical trials suggest that each 1 mg/dl (0.026 mmol/l) increment in LDL cholesterol causes an increase in coronary risk of 1%. Epidemiological observations have also shown an increase of 2-3% in risk for each 1 mg/dl (0.026 mmol/l) decrease in HDL cholesterol (Mensink & Katan, 1992).

In addition, plasma apolipoprotein A-I, the major protein of HDL, is a good negative predictor for coronary heart disease (Maciejko et al., 1983; Fruchart, 1990). Csazar et al. (1993) in their study of two large ethnically different populations of diabetic patients found that diabetes-induced elevation of Lp(a) might contribute to the increased risk for coronary heart disease in the diabetic population (Csazar et al., 1993).

Ruiz et al. (1994) have also demonstrated a positive and independent association between elevated Lp (a) levels and coronary heart disease in 71 NIDDM patients.

Recently, James et al. (1995) in the study of 500 people with NIDDM and IDDM noticed that Lp(a) concentrations greater than 30 mg/dl in these groups were independently associated with vascular disease. Also Hiraga et al. (1995) studied 221 patients with NIDDM who were followed for 2.2-3.1 years. They found that those patients who had higher Lp(a) concentration had a higher incidence of CVD.

In addition, in patients with coronary heart disease, total plasma ApoB levels are often significantly elevated in the presence or absence of a reduction of total plasma ApoA-I (Douste-Blazy and Kloer, 1989).

Risk factors for coronary artery disease may actually increase when patients with NIDDM consume a low-fat, high-carbohydrate diet (Reaven, 1988). The Seven Countries Study is one of the best known example of investigations concentrating on the magnitude of the association between serum cholesterol and heart disease, across several different communities (Keys, 1970). The diet and its possible effect on the death rate were followed during periods of 10 years and 15 years (Keys et al., 1986). The results showed a high correlation between the average percentage of dietary energy from saturated fatty acids and the 10-year incidence of coronary heart disease and the death rate from coronary heart disease. A further five years of follow-up also confirmed these results. The death rate was negatively related to proportion of dietary energy derived from monounsaturated fatty acids, but was unrelated to that obtained from polyunsaturated fatty acids, proteins, carbohydrates and alcohol (Keys et al., 1986).

2.6 Blood pressure and NIDDM:

The prevalence of hypertension is notably higher among NIDDM individuals than the among the general population. In humans, hypertension occurs approximately twice as frequently in those with diabetes as in non-diabetic individuals (Krolewski et al., 1985).

Hypertension is important in the diabetic population because of its association with both macrovascular and microvascular complications (Best, 1992). This view is supported by a number of studies including the Multiple Risk Factor Intervention Trial (MRFIT), in which high blood pressure was found to be a significant predictor of cardiovascular mortality in diabetic individuals (Stamler et al., 1993).

About 50% of patients with NIDDM are hypertensive. Hypertension may be due to increased total peripheral resistance or increase in cardiac output or both. Increased total peripheral resistance may be the result of increased arteriolar constriction (Vander et al, 1994b). About 95% of people with hypertension have essential hypertension (primary hypertension) in that the cause of hypertension is unknown (Ross, 1990; Durrington, 1995). The prevalence of hypertension in patients with diabetes is 1.5 to 2 times greater than in the general population (Best, 1992).

Moreover, hypertension becomes more prevalent with a longer duration of diabetes. The Framingham Heart Study showed that as many as 50% of diabetic people have some degree of elevated blood pressure; a positive correlation between the incidence of both diabetes and hypertension, and obesity was also detected (Kannel and McGee, 1979).

Even higher prevalence rate were recorded in the San Antonio Heart study, in which 85% of diabetic subjects were found to be hypertensive by the age of 50 years (Mitchell et al., 1990). Also in this population, 80% of obese subjects had abnormal glucose tolerance and were hypertensive.

Istfan et al. (1992) have reported that obese hypertensive subjects had greater insulin resistance compared with obese non-hypertensive subjects. It is thought that the relationship between primary hypertension and insulin resistance is independent of obesity (Pollare et al., 1990).

Hypertension is common in newly diagnosed patients with NIDDM. The Hypertension in Diabetes Study (HDS) found that 39% of patients (35% males and 46% females) were hypertensive as early as 2 months after diagnosis (The Hypertension in Diabetes Study, 1993).

An abnormal lipoprotein profile, similar to the dyslipidemia of diabetes (hypertriglyceridemia and a low level of HDL-c), has also been demonstrated in patients with essential hypertension (Shieh et al., 1987), suggesting that a number of patients with essential hypertension, in fact, have hypertension associated with the insulin resistance syndrome (Stern, 1996).

In relation to the effect of diet on blood pressure, Rasmussen et al. (1993), using 24-h ambulatory BP monitoring, reported lowering of daytime BP with a high monounsaturated fat diet compared with a high carbohydrate diet. In contrast, Straznicky et al. (1993) using 24-h monitoring, reported a slight but significant lowering of mean BP with a high carbohydrate diet compared with high monounsaturated fat diet. These differing results may reflect patient heterogeneity.

2.7 Smoking and NIDDM:

Smoking is a risk factor for ischaemic heart disease (IHD) (Meade et al., 1987) and may increase the incidence of NIDDM (Bjorntorp, 1988; Pederson, 1989; Rimm et al., 1993). Smoking is epidemiologically related to some risk factors including insulin resistance (Facchini et al., 1992) and abnormalities in plasma lipoproteins, particularly decreased HDL-C (Criqui et al., 1980, Wilson et al., 1983). The correlation between smoking and decreased HDL-C has been confirmed by previous studies (Craig et al, 1989; Maede et al., 1991; Muscat et al., 1991). However, some researchers have rejected the independent effect of smoking on HDL-C after correction for lifestyle factors (obesity, lack of exercise, alcohol intake, etc.) (Wallentin and Sundin, 1985, Manttari et al., 1991).

Cigarette smoking favours the development and progression of diabetic nephropathy. Several large prospective cohort studies have reported that the relative risk for all-cause mortality is approximately twice as high for smoking compared to non-smoking diabetic patients. Stopping smoking is probably the most cost-effective risk factor intervention for diabetic patients (Muhlhauser, 1994).

Plasma HDL-C rises within 2-8 weeks to the normal range after smoking cessation (Fortman et al., 1986; Stamford et al.; 1986; Moffatt, 1988). Some investigators has questioned whether increased HDL-C level is due to stopping smoking or dietary changes (Quensel et al., 1989, Maida and Howlett, 1990). Quensel et al. (1989) believe that the HDL had changed because of the increased dietary fat and carbohydrate intake after stopping smoking.

Smokers have been reported to have lower plasma concentration of HDL_2 (Shennan et al., 1985), HDL_3 (Haffner et al., 1985, Moriguchi et al., 1990), and HDL_2 and HDL_3 (Robinson et al., 1987).

Craig et al. (1989) also have found that apo A-I concentration is low in smokers. In addition, plasma levels of TG (Simons et al., 1984), VLDL (Freedman et al., 1986) and LDL-C have been reported to be higher in smokers compared with non-smokers (Craig et al, 1989, Freedman et al, 1986, Muscat et al, 1991). Smokers also have transient increases in heart rate and blood pressure (Freestone and Ramsay, 1982).

Although the pathogenic link between these changes and smoking is not well understood (Frati, 1996), there is no doubt that diabetic patients who smoke have higher mortality rates due particularly to peripheral occlusive arterial disease (Klein et al., 1989; Morrish et al., 1991; Palumbo et al., 1991; Muhlhauser, 1994).

2.8 Physical Activity and NIDDM:

Sedentary lifestyle is related to increased coronary atherosclerosis and physical activity decreases the risk of coronary atherosclerosis (Paffenbarger et al., 1986, Blair et al., 1989). Regular exercise enhances insulin sensitivity in people with non-insulin-dependent diabetes mellitus (King et al., 1988; Simopoulos, 1994; Braun et al., 1995), reduces blood sugar and lipid levels, lowers blood pressure, reduces body fat, and leads to weight loss (Bush, 1991; Simopoulos, 1994; Barnard et al., 1994; Braun et al., 1995).

There have been various recommendation on the amount of physical activity needed to improve glycemic control and insulin sensitivity. Exercise has been recommended three times per week for 20-45 minutes at 50-70% of maximum capacity (Howard and Howard, 1994). Currently the major recommendation is 4-5 times a week at 50% VO2 (max) for 45-60 minutes (Horton, 1996)

2.8.1 Effects of physical activity on lipids and lipoproteins:

It is well accepted that physical activity improves lipoprotein metabolism (Huttunen et al., 1979, Berg and Keul, 1985). Researchers have reported a decrease in plasma LDL (Wood et al., 1977, Goldberg et al., 1984, Hurley et al., 1986) and TG concentration following regular exercise (Wood et al., 1977, Farrell and Barboriak, 1980). With perseverance, exercise leads to a fall in VLDL-TG and LDL-C levels (Thompson et al., 1988, Wood et al., 1988). These changes are due to increased LPL activity and plasma TG clearance (Thompson et al., 1988). Moreover, exercise increases the plasma concentration of HDL-C fractions (Gordon and Rifkind, 1989, McHenry et al., 1990).

Exercise programs with moderate intensity seem to modify the effects of a hypocaloric, fat-restricted diet on HDL-C (Taylor and Ward, 1993).

2.8.2 Effects of physical activity on carbohydrate metabolism:

In low to moderate intensity exercise, rapid adjustment in the supply of energy is the result of fall in plasma insulin, and steady or increased glucagon production to maintain euglycemia. Hepatic glucose production in moderate exercise is regulated mainly by the glucagon / insulin ratio (Purdon et al., 1993).

In contrast, intense exercise is related to a marked stimulation of hepatic glucose production with resulting hyperglycemia, marked increases in plasma catecholamines, and moderate hyperglucagonemia. The hyperglycemia increases in recovery and is accompanied by hyperinsulinemia (Purdon et al., 1993).

King et al. (1988) have reported that the reversal of increased insulin action that occurs within few days after stopping exercise is due to a decrease in insulin sensitivity, not to a decrease in insulin secretion.

2.9 Alcohol Consumption and NIDDM:

Alcohol (ethanol) is a source of energy and it also has potent pharmacological and biochemical properties that may influence other aspect of nutrition and metabolism (Mitchell, 1990).

Ethanol is metabolized in the liver to form acetaldehyde, which in turn is oxidized to acetate. Acetaldehyde (in excess) may cause liver damage by decreasing mitochondrial functions. In heavy alcohol consumers, alcohol becomes a preferred fuel for the liver and displaces fat as a source of energy. Oxidation of fatty acids is decreased and the production of TG is increased. If this process continues a fatty liver may develop (Lieber and Pignon, 1989; Howard and Howard, 1994).

In diabetes, alcohol influences glycemic control, and plasma lipid levels and contributes to excess caloric intake (Griver and Henry, 1994). A number of studies have reported that alcohol consumption increases plasma TG concentration

(Ostrander et al., 1971, Castelli et al., 1977; Pagnan and Bonanome, 1993). The consumption of enough ethanol to give a blood concentration over 1 g/L and mild intoxication results in a rise in TG concentration in the plasma of normal subjects (Lieber and Pignon, 1989). Ethanol consumption leads to an increase in VLDL synthesis and limitation of alcohol may markedly decrease plasma TG concentrations (Ginsberg et al, 1974, NIH Consensus Conference, 1984).

There is a positive correlation between alcohol intake and HDL-C levels in population studies (Moore and Pearson, 1986). Several studies have found alcohol consumption may lead to an increase in HDL level (Haskell et al, 1984; Sillanaukee et al., 1993; Durrington, 1995). ApoA-I and apoA-II are increased by alcohol (Camargo et al, 1985). Studies have reported that alcohol-related HDL-C elevation may be due to increased hepatic apoA-I synthesis (Taskinen et al., 1987, Okamoto et al., 1988).

A reduction in LDL-C level may be seen in alcohol drinkers (Taskinen et al, 1982). The modification of LDL-apoB by acetaldehyde (the first metabolite of ethanol) increases the catabolic rate of LDL particles in the rat, resulting in lower LDL-C levels (Kesaniemi et al., 1987; Savolainen et al., 1987). It can be an explanation for decreased LDL-C concentrations in alcohol users. Alcohol consumption also increases the blood pressure (MacMahon, 1987; Rivellese et al., 1980; Pagnan and Bonanome, 1993).

There have been various recommendations on alcohol. Recent guidelines suggest that alcohol should be consumed in moderation, not more than 2 equivalents of alcoholic beverage (20 g ethanol) once or twice a week (Griver and Henry, 1994).

More recent guidelines still are less restrictive, with an allowance up to 4 units / day (ie 40 g/day) in men, 2 units / day in women, with an alcohol - free day each week (ADA, 1997).

2.10 Sodium intake and NIDDM:

Sodium intake should be limited in NIDDM patients due to the frequent development of hypertension and nephropathy. Therefore the recommended maximum intake of sodium for NIDDM is 1000 mg per 1000 kcal, and it should not exceed 3000 mg/day (Griver and Henry, 1994).

2.11 Diet intake methodology:

Campbell et al. (1989 & 1994) asked the individuals in their studies to complete 4 day food records, including 2 weekend days. Each individual was trained by a dietitian in accurate food recording. Food measures and food models were used. The recording of brand names and the details of cooking methods were emphasized to aid accuracy. The dietitian also placed emphasis on the impersonal, non-judgmental calculation of dietary intake by computer. The 4 day dietary records were analysed using the "Diet 1" computer program (Xyris software, Queensland), based on the NUTTAB food composition data base. As Campbell et al. (1989 &1994) encountered few problems with their chosen diet intake and nutrient analysis methodology, successful replication of their methodology would seem possible.

2.11.1 Measuring dietary compliance:

Campbell et al. (1994) asked the individuals involved in their study to complete a visual analogue questionnaire concerning dietary preferences. They also analysed the 4 day food records using the diet 3 computer program, and compared the results

obtained with the composition of prescribed diets. Sarkkinen et al. (1994) also measured the degree of dietary adherence to the diet using repeated dietary records.

Delahanty and Halford (1993) used a diet behaviour questionnaire which asked the individuals involved in their study to report on methods used to implement diet, adherence to meal plans and the management of expected changes in dietary intake.

As the individuals involved in the study by Garg et al. (1994) were supplied all their food, dietary compliance was assessed by commenting on the unconsumed food returned to the researchers. Dietitians involved in the study also conducted weekly interviews with the individuals.

In spite of this, many studies have demonstrated that most people with NIDDM do not achieve the treatment goals recommended by the American Diabetes Association (Consensus Statement, 1995).

2.11.2 Individual dietary prescription:

In order to supply diets isocaloric to normal intake, Campbell et al. (1994) based the diets of the individuals involved in their study on an initial nutritional analysis obtained from a 4 days food record. Garg et al. (1994) calculated the energy requirements of the individuals involved in their study using the Harris Benedict Equation, and multiplying it by an activity factor. High monounsaturated fat diets implemented by Bonanome et al. (1991), Sarkkinen et al. (1994) and Lerman-Garber et al. (1994) were all isocaloric. In all the above studies subjects were instructed not to change their physical activity.

2.11.3 Dietary compliance by individuals with NIDDM:

Diet plays an integral role in the control of diabetes and yet every survey shows that most people with diabetes in practice do not follow dietary instruction

accurately. Possible reasons for failure of compliance are at least three fold. First, perhaps not enough time is spent on explaining the purpose of the diet and what can be achieved. Second, the diet recommended may be too remote from the habits, customs and capabilities of the diabetic concerned. Third, the diet sheet information may be too detailed and complicated (Bloom & Ireland, 1992)

It is interesting that in one study subjects who consumed a high monounsaturated fat diet felt more active and energetic than the group on a high carbohydrate diet (Walker et al., 1995).

CHAPTER THREE

METHODOLOGY

3.1 Ethics approval:

The Human Research Ethics Committee at the University of Wollongong granted ethical approval.

3.2 Study population:

Illawarra residents with a diagnosis of NIDDM and registered with the Diabetes Education Centre in Wollongong.

3.3 Sample selection:

Subjects were randomly selected (age range: 30 - 50 years old) from those with a diagnosis of NIDDM from a list of about 2000 names of diabetic patients who had been referred to the Diabetes Education Centre in Wollongong; eg. names were numbered and every 10th name was selected and contacted. Of those who were contacted, 50 were selected who were eligible for study criteria and were willing to participate. All subjects had received education in an orthodox low fat high complex carbohydrate diet at the time of referral, in most cases shortly after diagnosis of diabetes. They were initially contacted by a letter which asked them if they would like to volunteer to participate in the study (Appendix 1). Subjects were randomly allocated either to a high carbohydrate low fat diet or a diet high in monounsaturated fat to be followed for a period of one year. Individuals were asked separately if they would participate in euglycemic clamp tests of insulin sensitivity.

Twenty seven accepted. Subjects were excluded from the study if they were using insulin or had a history of renal disease or acute liver disease.

Following an agreement by the subject to participate in the study, an appointment for the initial baseline consultation was made. After initial introductions, the purpose of the consultation, and the confidentiality of the information provided was explained to the subjects and the consent forms (Appendix 1) were signed.

3.3.1 Experimental Design:

The study was a randomised controlled non-blinded trial; a comparison over one year of the effect of a diet rich in canola oil (which contains 63% n-9 monounsaturated oleic acid and 10% n-3 α-linolenic acid, and 20% n-6 linoleic fatty acids), with a control diet, a low fat high complex carbohydrate diet, on indicators of diabetes control in people with NIDDM. Though analysis of laboratory test was blinded, blinding, was otherwise judged impractical for investigators and subjects.

3.3.2 Diet composition:

3.3.2.1 High carbohydrate low fat diet (Control group):

Participants were asked to follow standard dietary guidelines for NIDDM, guidelines which were in use in the Illawarra Area Health Service Diabetes Education Centre. Guidelines were for a low fat, high complex carbohydrate diet based on recommendations made by American Diabetes Association (1994) and other bodies. At least 50% of total energy intake should be carbohydrate, and less than 30% of total energy as fat (less than 10% of energy as saturated fat and less than 10% as polyunsaturated fat), and less than 300 mg per day cholesterol intake. The intake of fruits and vegetables and grain products (and thus dietary fibre) was encouraged, and alternatives to a high meat and dairy product intake were suggested.

In the first 3 months, the dietitian consulted with the participants once a month and had regular phone contact throughout the year. Also the dietitian consulted with the participants at 6 and 12 months time as well.

Each individual was given intensive practical instruction by the dietitian, and given written information on the high carbohydrate low fat diet, detailing the specific amounts of food to be consumed, and recommendations on choosing from the main food groups; a list of the foods which could be consumed freely was also supplied.

3.3.2.2 High monounsaturated fatty acid diet (HMUFA group):

In the HMUFA diet patients were asked to consume an isocaloric diet containing 40% of energy intake as carbohydrate and 38% as fat (preferably canola oil which contains about 20% n-6 linoleic acid, 10% n-3 α-linolenic acid and 63% n-9 oleic acid). The proposed diet had a high dietary fiber content and less than 300 mg cholesterol intake per day, all in the setting of a Mediterranean cuisine (Campbell et al. 1994).

Since the BMI varied between subjects, the dietary prescription for each subject was calculated individually. Estimates of energy requirements were based on the Harris Benedict equation informed by consideration of the usual dietary intake (Garg et al. 1994). Energy intake was adjusted as necessary to maintain constant body weight (Sheard, 1995).

Harris Benedict Equation:

BASAL ENERGY REQUIREMENTS (BEE):

for men = 278 + (57.5 x **W**) + (20.9 x **H**) - (28.3 x **A**) kJ / day.

for women = 2741 + (40 x **W**) + (7.7 x **H**) - (19.6 x **A**) kJ / day.

ENERGY REQUIREMENTS = BEE x activity factor x injury factor;

where.....(**W** = weight in kg
H = height in cm
A = age in years) (Dietitian's Pocket Book, 1994 p 36)

If the reported dietary intake of subjects did not match their calculated energy requirements, then estimates of energy requirements were based on the mean of the Harris Benedict Equation energy requirements and reported dietary intake.

The grams of fat required to provide 38% of the total energy intake were calculated, using Atwater factors (eg: 1 gram of fat provides 37kJ). The fat allowance required to meet 38% of total energy was divided by 5 (one serve of monounsaturated fat was equivalent to 5 g of fat) after subtracting 10 grams of fat allowance for meat. Subjects were required to consume the specified number of serves of monounsaturated fat per day. Sources of monounsaturated fat stipulated on the diet sheets provided included Canola oil, olive oil, Canola margarine, olive margarine, Canola mayonnaise, avocado, peanut butter, olives and nuts (pecans, hazelnuts, cashews, peanuts, almonds, macadamias, and pistachio).

Simple substitutions were suggested for the individual's usual diet, based on the practical tips developed by Campbell et al. (1994), to help individuals to prepare a diet rich in monounsaturated fat (Appendix III).

Individuals were advised preferably not to include alcohol in their diet, and if they drank, only to drink in moderation (less than 30 grams per day) (DAA, 1990).

Each subject was also given a generic meal plan, and a sample meal plan to demonstrate the incorporation of foods containing monounsaturated fat to the diet (Appendix III). Detailed instructions were given at the beginning of the study, and assistance was given throughout the study to participants in both groups, to assist them to reach the dietary goals. A similar amount of time was devoted by the dietitian to dietary instruction for both groups. Each subject was provided with diet sheets appropriate to their specific dietary regimen (Appendix III). In order to prevent weight loss or gain subjects were instructed to maintain present activity levels.

3.4 DATA COLLECTION:

3.4.1 Demographic:

Fifty male and female (M=26, F=24) NIDDM patients, average age: 45 ± 0.9 (±SEM) were randomly allocated to two diet groups.

Demographic information included previous medical history, duration of diabetes, medication used, family history of diabetes, heart disease, details of smoking and alcohol consumption, employment status, marital status.

At three and twelve months time the following information was checked: changes in regard to smoking, alcohol consumption and medication intake.

Baseline food records returned by the subjects were checked to ensure accuracy (amounts of foods consumed / description of food / food preparation techniques) (Campbell et al. 1994).

3.4.2 Diet information:

3.4.2.1 Food record:

Participants were informed that they would be required to keep a 4 day food record on 4 occasions (baseline / 3 months / 6 months / 12 months). Food records were to be completed on four consecutive days: 2 weekdays and 2 weekend days as close to the next appointment as possible (Appendix III). If the participants were shift workers they were instructed to record their dietary intake on 2 weekdays and 2 days off.

Participants were informed that their dietary intake would be treated in a non judgmental manner, and would be analysed confidentially via a computer programme. The techniques for keeping an accurate food record were explained using hypothetical examples. The importance of not changing or under-reporting dietary intake was emphasised.

3.4.2.2 Consultations:

At the first consultation participants completed a diet information sheet detailing their usual dietary intake. A broad diet history (Appendix III) was also collected in order to obtain baseline dietary information to be used in the following dietary prescription. Goal areas included the amount and type of dietary fat, type and serves of milk products per day, serves of pasta / rice / starchy vegetables and bread per day, serves of fruit and vegetables per day, beverage consumption (especially alcohol), food preparation techniques, seasoning / sauces / dressings added to food, use of salt. The frequency of consumption of cakes / sweets / pastries / biscuits, take away foods, snack foods, restaurant food was also targeted. Subjects were specifically asked the frequency of avocado, peanut butter, olive oil and nut consumption, all sources of monounsaturated fatty acids. Subjects were also asked

to provide information regarding: prior dietetic consultations, knowledge of their blood glucose level ranges, country of birth (to ascertain cultural background), who prepares the food at home and for how many, physical exercise habits (type, duration, frequency and intensity if applicable).

Food models were used in consultations with the subjects allocated to follow the high monounsaturated fat diet. Other prompts included bottles of monounsaturated oils, a variety of nuts containing monounsaturated fat, monounsaturated margarine, monounsaturated oil cooking sprays and milk containing monounsaturated fat ("Farmers Best").

To ensure the accuracy of food measures (especially the fat quantities), standardrized cup and spoons measures were provided to subjects. Also diagrams of the size and thickness of different types of meats (red meat, fish, pork, and chicken) were supplied for the participants. The use of scales where appropriate was encouraged.

In the second consultation an open ended questionnaire (previously implemented on the telephone) was discussed in an interview with each subject (Schlundt et al. 1994). The food records of each subject were checked for accuracy as in the previous consultation. Subjects were then provided with a food record to complete and return in the third consultation (6 months time).

In the third consultation (6 months) with the dietitian, their food records were checked. This consultation was mainly to check that the participants were following their prescribed diet. Subjects were also provided with a food record to complete and return it in the fourth consultation (12 months time).

In the last consultation (12 months), the four days food records were checked for accuracy as in the previous consultations. Participants were also asked to complete the "diet acceptability" form.

Overall participants had frequent continuous communication: either by phone, letter, or face to face consultation with the dietitian throughout the 12 months of the study.

3.4.2.2.1 Questionnaire format:

Open ended questionnaires on factors which influence dietary compliance were given both to the subjects following the high carbohydrate low fat diet, and the subjects following the high monounsaturated fat diet (Campbell et al. 1994, Schlundt et al. 1994), at the end of the three months. The questionnaires for the two subject groups were not identical. Both questionnaires targeted the main problems encountered by the subjects in adhering to their allocated diet, and asked whether subjects had been able to consume specific food items in recommended amounts. The questionnaires are included in Appendix III.

One area in which the questionnaires differed was in the discussion of dietary fat consumption. Individuals following the high carbohydrate low fat diet were asked whether they thought that they were eating fats and oils in limited amounts. They were also asked whether they were avoiding specific foods which are high in fat, and the reasons why, or why not they were avoiding high fat foods. Individuals following the high monounsaturated fat diet were asked questions on the ease of consuming the amount of foods containing monounsaturated fat that they were asked to consume. They were also asked wether they had managed to consume "Farmers Best" milk, and avoid specific foods containing saturated and polyunsaturated fats.

Both subject groups were asked questions on: (1) changes in dietary consumption following dietary intervention, (2) whether they thought that their allocated dietary regimen was palatable and enjoyable, (3) whether or not they had any food preparation problems, (4) whether they thought that they would be able to continue eating in a similar fashion on a long term basis (5) whether the cost of following their allocated dietary regimen was a problem, and (6) whether the information (diet) sheets provided to them were sufficient, and if not what further information should have been provided. In order to obtain any additional information which may have been overlooked both questionnaires concluded with the question, "can you tell me anything else to help us in understanding what it is like to be on this diet?"(Appendix III)

3.4.2.2.2 Diet acceptability form:

Questions relating to diet acceptability were asked at twelve months, and a response on a scale from 0 to 10 was sought (Appendix III). The participants were asked to give their opinion on aspects of their diet: `ease of preparation', `cost', `taste', `satisfaction', `variety', `adherence', `convenience for the family'. They also had opportunity for additional comments on their diet. (Campbell et al, 1994)

3.5 Measurements:

3.5.1 Anthropometric measurement:

All anthropometric measurements were carried out in accordance with the WHO standards (WHO, 1987).

Subjects were asked to remove all clothing except underwear, and change into a white hospital gown. Body weight was determined to the nearest 0.1 kg using Mercury balance scales. Body height was measured to the nearest 0.1 cm by using

a wall-mounted stadiometer. The subjects stood with their back against the wall and with feet together. Arms were outstretched maximally at the level of the shoulders, with palms facing forwards (Fidanza, 1991). Body mass index (BMI) was calculated by dividing weight (in kilograms) by height squared (in meters) (Weigley et al, 1994).

3.5.2 Blood pressure:

Blood pressure was measured on the right arm in the sitting position, after five minutes of rest, using a standard mercury sphygmomanometer- two measurements were taken and averaged. Systolic blood pressure was taken to be the appearance of korotkoff sounds, and diastolic at their disappearance (phase 5) (O'Brien and O'Malley, 1991).

Mean arterial pressure (MAP) was calculated by adding the sum of two thirds of the diastolic pressure and one third of the systolic pressure (Konen et al, 1993).

3.5.3 Fat percentage and skinfold measurement:

Waist and hip circumference measurements were obtained using a flexible measuring tape. The waist was measured as the narrowest circumference between the lower costal margin and the iliac crest in the standing position. The hip was measured as the maximum circumference at the level of the femoral trochanters (Banerji et al, 1995). Regional fat distribution was assessed by dividing the waist circumference by the hip circumference in order to obtain the waist-to-hip ratio (WHR).

Skinfold thickness were measured with metal Harpendon skinfold callipers. All skinfold measurements were measured on the right side of the body. Biceps,

triceps, subscapular and suprailiac skinfold sites were used (Bray, 1985). Three skinfold measures were taken at each site and averaged to assess body fat levels (Harpendon skinfold callipers, Crymych, United Kingdom). Biceps skinfold measurements were taken at the biceps muscle midpoint, above the centre of the antecubital fossa. Triceps skinfold measurements were taken midway between the inferior border of the acromion process and the tip of the olecranon process, in an exact line between the point of the elbow and the acromion process. Subscapular skinfold measurements were taken from an area marginally below the tip of the scapula, at a 45° degree angle vertically from the spine. Suprailiac skinfold measurements were ascertained from an area slightly above the superior iliac crest in the midaxillary line. The readings at any one site were within 5% of each other (Steinbeck, 1992). Body density was calculated using the Durnin and Womersley equations (Gibson, 1990). Percent body fat was determined from body density as the average of the values obtained using the Siri equations (Gibson, 1990).

All anthropometric measurements were performed at base line, three and twelve months by the same observer (Zeman, 1991).

3.5.4 Euglycemic clamp study:

Insulin sensitivity was measured by the euglycemic clamp technique (DeFronzo et al, 1979) at 0, 3, and 12 months by the same investigator. Commencing at 0800 h, after a 12 hours overnight fast, an intravenous catheter was inserted into an antecubital vein for infusion of glucose and insulin. Another cannula was inserted into a dorsal vein of the hand in the retrograde direction. To provide arterialized venous blood for sampling, the hand was warmed to approximately 50°C with a heating blanket (Bergman et al, 1987). This second cannula was flushed every time that blood was taken with isotonic saline and the first 1 mL of blood sample was subsequently discarded at each sample time. Purified porcine insulin

(Actrapid, Novo Nordisk Pharmaceuticalís, NSW, Australia) was infused as a 10-min priming infusion followed by a constant infusion of 40 $mU.m^{-2}.min^{-1}$ throughout the study. During the insulin infusion, glucose was maintained by a variable infusion of 10% dextrose.

The blood glucose concentration was clamped at 4.5-5.0 mmol/L for the last 60 minutes of the test, using a variable rate of glucose infusion, adjusted according to blood glucose measurements taken at 5-min intervals with a glucose analyzer (Yellow Springs Instruments, Yellow Springs, Ohio USA). In this euglycemic steady-state condition the glucose infusion rate is equal to the glucose uptake rate by the body, and is therefore a measure of whole body sensitivity to insulin (De Fronzo et al., 1979; Borkman et al., 1991; Borkman et al., 1993).

The amount of glucose metabolized during the euglycemic clamp is the sum of infused glucose plus endogenously produced glucose. Since 95-100% suppression of hepatic glucose production occurs during the clamp study, any increase in the glucose infusion rate during a clamp study reflects enhanced peripheral glucose uptake rather than increased hepatic suppression (Yki-Jarvinen & Koivisto, 1983). Hepatic glucose production was not considered in the calculation of metabolized glucose (Schwenk et al, 1990).

The quantity of glucose infused during the final 60 minutes, corrected for body-surface area, provided an index of the insulin sensitivity of the whole body, expressed in terms of the number of milligrams of glucose infused per square meter per minute ($mg.\ m^{-2}.min^{-1}$) (Bergman et al, 1987; Borkman et al., 1993).

3.5.4.1 Pilot study:

The euglycemic clamp method was piloted with two volunteers (female and male) at the beginning of the study.

3.5.5 Indirect Calorimetry:

The technique of indirect calorimetry is based on the measurement of gas exchanges, using an open-circuit ventilated hood systems and large respiration chambers. By measuring oxygen consumption (VO2), carbon dioxide production (VCO2) and urinary nitrogen (N) in most cases, it is possible to calculate carbohydrate, fat and protein oxidation, and the nutrient balance, if food intake is known. It is also possible to measure the respiratory quotient (VCO2/VO2). To avoid short-term effect of changes of the CO2-bicarbonate pool on the calculation of the substrate oxidation rate, it is recommended to carry out continuous measurement of indirect calorimetry, and to determine average values of VO2 and VCO2 for periods of 5 min or more. Indirect calorimetry also calculates respiratory quotient (RQ) which is the synthesis of glucose from amino acids or fat oxidation. In summary, indirect calorimetry is the method by which the type and rate of substrate utilization and energy metabolism are estimated in vivo from gas exchange measurements (Ferrannini, 1988).

Indirect calorimetry was performed with a computerized flow-through canopy gas analyzer system (Deltatrac, Datex, Helsinki). This device has a precision of 2.5% for O_2 consumption and 1.5% for CO_2 production (Haffner et al, 1995). The machine was calibrated every day, prior to use, according to the manufacturer's instructions. On the day of the experiment, gas exchange (O_2 consumption and CO_2 production) was measured for half an hour before the start of the clamp test and also for an hour during the last hour of the clamp study. The values obtained during the first 10 min of the data set were discarded, and the mean value of the remaining 20-

min data was used for calculations. Protein, glucose, and lipid oxidation were calculated according to Ferrannini et al. (1987). The fraction of carbohydrate non-oxidation during the glucose clamp studies was estimated by subtracting the carbohydrate oxidation rate (determined by indirect calorimetry) from the glucose infusion rate (determined by the euglycemic clamp) (Haffner et al, 1995).

Subjects were instructed to fast and abstain from exercise for 12 h before the test. Participants rested for 20 min before the measurements were taken, reclined in a pleasant, semi-darkened, quiet room with only the researcher present. Respiratory quotient (RQ) and exchange equation (REE) were computed by continuous measurements of oxygen consumption and carbon dioxide production at 1-min. intervals, 30 minutes before starting the clamp and over the last 60 minutes of the clamp study.

3.5.6 Body Mass Index:

The Body Mass Index (BMI) is a valid measure of body mass and adiposity and defined as weight (kg).height $^{-2}$ (m^{-2}) (Weigley, 1994).

3.5.7 Biochemical and lipid measurement:

A 12-h fasted venous blood sample was collected from participants by the investigator for the biochemical measurements. Blood for glucose and insulin was collected into evacuated serum gel tubes (Serum Gel S/7.5 mL, Monovette, Sarstedt, Germany). Blood for HbA1c and lipid analyses was collected into dipotassium EDTA-containing (1.6 mg/mL) tubes (Monovette, Sarstedt, Germany).

Blood was centrifuged immediately at 2300 rpm for 20 minutes in a swinging bucket centrifuge (Sorvall Instruments, Du Pont, DE, USA). Plasma was kept at

4°C if lipid analyses were to be performed on the day or stored at -85°C, in 2 mL snap-top plastic tubes until analyzed. Other blood samples (citrated plasma and serum) had analyses performed in the Pathology Laboratories, Illawarra Regional Hospital.

Fasting blood glucose (FBG) was measured colorimetrically by the glucose oxidase / peroxidase and bromcresol method, on a Ektachem 700XR analyzer (Kodak, NY, USA) using the Kodak Ektachem Clinical Chemistry Slide (GLU) (Trinder, 1969).

(reference range in non-diabetic adults: 3.8-7.8 mmol/L)

Fasting blood insulin was measured by microparticle enzyme immunoassay (MEIA) based on the Travis (1980) method on an Abbot IMX analyzer (Abbott Diagnostics, IL, USA). In the IMX insulin assay, an antibody-insulin complex is formed by incubating the patient's serum sample with coated microparticles. Then the anti-insulin:alkaline phosphatase conjugate is added to the antibody-insulin complex and binds to it. The substrate, 4-Methylumbelliferyl Phosphate, is added to the complex and the end product of the reaction (the fluorescent product) is measured by the MEIA optical assembly (Abbott Diagnostic, 1994). The IMX insulin assay shows no cross-reactivity with proinsulin (Abbott Diagnostic, 1994).

The IMX Insulin Calibrators contain insulin (human) prepared in buffer at six different concentrations (0, 3, 10, 30, 100, and 300 μU/mL).

The IMX Insulin Controls contain insulin (human) prepared in buffer to yield the following concentration ranges.

Bottle	Insulin concentration (μU/mL)	Range (μU/mL)
L	8	6-10
M	40	32-48
H	120	96-144

(reference range in non-diabetic adults: <20 mIU/L)

HbA1c was measured by high performance liquid chromatography (HPLC), on a Pharmacia Mono S HR 5/5 column (Pharmacia LKB Technology, Almeda, CA) (Bio-Rad Haemoglobin A1c Micro-column Test Instruction Manual, 1990).

Indicators used in this laboratory are:

> 10%	Poor degree of glucose control
9-10%	Fair " " "
8-9%	Good " " "
7-8%	Excellent " " "
< 6% (4.2-5.9 %)	Non-diabetic level

C-peptide plasma levels were determined by radioimmunoassay technique (intra-assay coefficient of variation 6%, 0.33 nM) (Heding, 1975).
(reference range in non-diabetic adults: 0.5-3.0 ug/L)

3.5.7.1 Lipids and apolipoproteins in plasma (Appendix II):

Total cholesterol, free (non-esterified) cholesterol (FC), and triglycerides were measured on a Cobas-Fara automated centrifugal analyzer (Roche Centrifugal Analyzer, France).

Total cholesterol concentrations in plasma and different lipoproteins were measured by the CHOD-PAP method (a standard enzymatic colorimetric method) based on Siedel et al. 1983 and Kattermann et al. 1984) with reagents supplied by Boehringer

Mannheim, Germany (Q C materials: Precinorm L, Cat No: L 781827 and Precipath Cat No: L 128574). Cholesterol was determined by enzymatic hydrolysis with subsequent determination of the liberated cholesterol by colorimetry. The reaction product was read optically at 546 nm and compared to a known standard curve. For total cholesterol six reference points (254, 508, 1016, 2032, 4065, and 8130 µmol/L) and 2 quality controls, one low (range 3635-5585 µmol/L) and one high (7350-11250 µmol/L) (Roche Control Serum Lipid Cat No 2023624) were used. (Reference range of cholesterol for non-diabetic adults used in this laboratory: <5.2 mmol/L)

Triglyceride concentrations (TG) in plasma and lipoproteins were measured by Triglyceride GPO-PAP (an standard enzymatic colorimetric method) based on Allaine et al. (1975) and Bucolo and David (1973) with reagents supplied by Boehringer Mannheim, Germany (Q C materials: Precinorm L, Cat No: L 781827 and Precipath Cat No: L 128574). Enzymatic hydrolysis of triglycerides was determined with subsequent determination of the liberated glycerol by colorimetry. The reaction product was read optically at 500 nm and compared to a known triglyceride standard curve. (Reference range of triglyceride for non-diabetic adults used in this laboratory: <1.8 mmol/L)

ApoA-I, and ApoB concentrations were measured in fresh plasma on a Cobas-Fara by Apolipoprotein B, T antiserum (standard immunoturbidimetric method) based on Becker et al, 1968, Thomas, 1978, and Dati et al, 1989 (Roche Diagnostics, France) (Appendix II). Turbidity of the antigen-antibody reaction was measured at 340 nm. As quality control (QC) for apo B and apo A-I, the apolipoprotein T Control (art. 0730696, Roche Diagnostics, France) was used.

Reference range for non-diabetic adults used in this laboratory:

	ApoA-I	ApoB
Female	.15-2.20 g/L	0.60-1.50 g/L
Male	1.15-1.90 g/L	0.70-1.60 g/L

Isolation of VLDL and IDL:

Isolation of lipoproteins was performed by density gradient ultracentrifugation at 4°C using 100.3, 100.4 or 50.3Ti fixed angle rotors (Beckman Instruments, Palo Alto, CA) and TLX, TL-100 or L8-70M ultracentrifuges (Beckman Instruments, Palo Alto, CA). Density ranges (g/ml) at which ultracentrifugation was carried out for the isolations were as follows: very-low-density-lipoprotein (VLDL) (0.96-1.006), intermediate density lipoprotein (IDL) (1.006-1.019) (Galton and Krone, 1991).

VLDL and IDL were isolated by sequential ultracentrifugation according a modified method of Havel et al. (1955). In 1 mL of normal saline (0.15M NaCl, density 1.006 g/mL), 2 ml of plasma was added in a 3mL bell-top quick seal centrifuge tube centrifuge tube (Beckman USA). The sample was then centrifuged at 25 minutes in a fixed angle rotor (TL 100.3; Beckman USA) in a benchtop ultracentrifuge (Beckmann Optima TLX; Beckmann USA). VLDL was isolated as the supernatant by slicing of the tube.

The infranatant was then adjusted to a density of 1.019 with solid KBr and KBr solution of density 1.019 g/mL up to 3 mls capacity of the centrifuge tube. Crystalline KBr was used to raise the solvent density KBr solutions were made in

100 ml of deionized water and contained 0.02% sodium azide. The amount of KBr to be added was calculated according to the formula of Hatch and Lees (1968).

After centrifugation at 100,000 rpm for 6 hours, IDL was isolated as the supernatant. The infranatant was adjusted to a density of 1.063 g/mL with solid KBr and KBr solution of density 1.063 g/mL.

Cholesterol ester (CE) was calculated in both VLDL and IDL as the difference between total cholesterol and free (non-esterified) cholesterol.

Non-esterified fatty acids (NEFA)

Concentrations of non-esterified fatty acids in plasma were measured by the WAKO enzymatic method (based on Duncombe, 1964, Novak, 1965, and Elphick, 1968) with reagent from Wako Pure Chemical Industries, Osaka, Japan. Oleic acid 1.0 mM was used as the NEFA standard solution (WAKO Pure Chemical Industries, Ltd, Japan).

The method relies upon the acylation of co-enzyme A (Co-A) by the fatty acids in the presence of added acyl Co-A synthetase (ACS). The acyl Co-A is oxidized by added acyl Co-A oxidase with generation of hydrogen peroxide. Then, hydrogen peroxide in the presence of peroxidase (POD) permits the oxidative condensation of 3-methyl-N-β hydroxyethylene aniline (MEHA). MEHA condenses with 4 amino antipyrine to form a purple colored product which can be measured colorimetrically at 550 nm. (Reference value in non-diabetic adult used in this laboratory: 0.20-1.08 mEq/L)

Isolation of HDL:

High-density lipoprotein cholesterol (HDL-C) and HDL-Apo A-1 was isolated from subjects plasma as the supernatant after precipitation of the lower density lipoproteins with a mixture of dextran sulfate and magnesium (Warnick et al., 1982).

LDL-C:

LDL-C was calculated according the modified Friedewald equation (all in mmol/L), (Samman and Truswell, 1993):

LDL-C (mmol/L) = Total cholesterol - HDL-C - TG / 2.18

3.5.7.2 Radiolabelling of HDL3:

Radiolabelled HDL3 (containing ^{3}H labelled cholesteryl ester) was prepared in the density range 1.13-1.21 g/ml as previously described by Alberts and Tollefson and stored at 4°C. The radiolabelling procedure resulted in the incorporation of >95% of the radioactive cholesterol into the HDL3 particles as determined by thin layer chromatography. Two preparations of [^{3}H] HDL (6.8×10^{3}) counts/min per nmol cholesterol) were used, for estimation of CETP activity on blood taken from participants at 0, 3, and 12 months.

3.5.7.3 Cholesteryl Ester Transfer Protein Activity Assays (CETPA):

CETPA was measured by utilizing whole plasma as the CETPA source as described by Abbey and Nestel (1994). CETPA was quantified as the percent transfer of radiolabelled cholesteryl ester from HDL3 to apo-B-containing lipoproteins. From

the plasma assay 100 µl of plasma was used incubated with [^{3}H] HDL$_3$ containing 0.05 mM cholesterol. Precipitation of apo-B-containing lipoproteins was carried out as for HDL-C determination. The transfer of radiolabelled cholesterol ester from HDL to apo-B containing lipoproteins was determined by measuring the decrease in radioactivity of the supernant. Counts were measured on a Tri-carb 1900 TR liquid scintillation analyzer (Packard Instrument Co., Meriden, CT) for 10 min following the addition of 10 mls of scintillation fluid (Ready Safe, Beckman, CA, USA).

Reference plasma and LPDP from a normolipidaemic volunteer was collected and stored at -85° C in small aliquots and assayed during all incubations. Non-specific cholesteryl ester transfer (<6.5%) was assessed using human LPDP in place of plasma.

3.6 Statistical Analysis:

Statistical analyses were performed using the JMP statistical package, version 3 (SAS Institute Inc., NC, USA). All statistical comparisons were two-tailed and $P<0.05$ was considered statistically significant unless otherwise indicated. Multiple comparisons were made, and in such circumstances statistical advice is usually to adopt a smaller α value (eg. $P \leq 0.01$). In the current study a single major outcome variable (insulin resistance syndrome) was examined, albeit with multiple indicators. In these circumstances the cautious use of $P \leq 0.05$ was thought appropriate, to test the central hypothesis. More stringent criteria should be used for unexpected data which are not consistent with or which do not logically accord with the central hypothesis.

Differences between mean baseline values and 3 and 12 months were analyzed using repeated measures analysis of variance (ANOVA). Paired student's t-test was

used to assess the significance of differences within the groups. The data are expressed as mean ± standard error of the mean (SEM). Variables were tested for normality using the Shapiro-Wilk W test. The Wilcox / Kruskal-Wallis test (Rank Sum) was used for data not consistent with the hypothesis of normality, to compare the changes between the groups.

Associations were determined using the Pearson correlation coefficient. Stepwise regression analysis was used to assess the independent relations between independent variables. The standardized ß coefficients and their significance are given for the variables remaining in the final models.

3.6.1 Diet analysis:

Nutrient analysis of the four day food records was carried out on Diet 1 Nutrient Calculation software (Xyris software, Queensland, Australia), based on the NUTTAB Food Composition Data Base (a compilation of Australian food analyses) (Lewis & English, 1990) and compared with the composition of the prescribed diets as an estimate of compliance. Foods not included in NUTTAB were separately coded (Campbell et al, 1994).

Multivariate analaysis of variance (ANOVA) for a repeated measures design was used to determine the changes between the measurements and the difference in the changes between the two groups (Laitinen et al., 1993; Sheard, 1995)

Mean nutrient intakes were calculated for the four days of both the baseline and post intervention food records. Mean intake for kilojoules (kJ), protein (g), fat (g), and carbohydrate (g) and dietary fibre (g) were calculated. The percentage distribution of polyunsaturated, monounsaturated and saturated fat was also calculated, along

with the percentage of total energy obtained from protein, carbohydrate, fat and alcohol.

To ascertain the occurrence or the significance of any changes, the percentages of total dietary fat along with the percentages of polyunsaturated, monounsaturated, and saturated fat, in relation to total fat consumption and carbohydrate were compared between and within the groups.

CHAPTER FOUR

RESULTS

This chapter given the results of the study at base-line, three months, six months, and twelve months. The significant differences or changes are shown with an asterisk (*) following the P value. There are four ways of showing the results:

1-Comparison between the two groups (eg. C3 and P3), using student t-test;

2- Comparison within the group (eg. P. and P3), using analysis of variance;

3- Comparison of the differences between the changes observed in the two diet groups in the specified time, using analysis of variance in repeated measurements. For example comparison of changes in measurements made at zero time and three months in two study groups (C3-0 vs P3-0), those on the control diet and the HMUFA diet. Changes for each individual were calculated (measurement at 3 months - measurement at 0 time) and the means and SEM of these changes are given. P value refers to the significance of the differences, in a comparison between the two groups of these means;

4-Correlation between variables and changes in variables during specified time, using multiple regression analysis. Some of these correlations are physiologically expected. Only some of the significant correlations are shown in the end of this chapter.

4.1 Past history and medication intake:

4.1.1 Past history:

Family history of diabetes: In the Control group 15 subjects (71%), and in the HMUFA group 16 subjects (76%) had family history of diabetes.

Hypertension: In the Control group, 7 (30%) subjects (duration of 3.17 ± 1.4 years) and in the HMUFA group 11 (40%) subjects (duration of 2.98 ± 0.96 years) had a history of hypertension.

Hypercholesterolemia: In the Control group, 6 (24%) subjects (duration of 0.7 ± 0.4 years) and in the HMUFA group 11 (48%) subjects (duration of 0.9 ± 0.4 years) had a history of hypercholesterolemia.

Heart related disease: In the Control group, 2 (8%) subjects and in the HMUFA group 4 (16%) subjects had a history of heart related disease; which were: angina pectoris (2), cardiomegaly (1), atrial fibrillation (2), and myocardial infarction (1).

Kidney related disease: In the Control group, 3 (12%) subjects and in the HMUFA group also 3 (12%) subjects had a history of kidney related disease; one had had nephritis, and five had had kidney stones.

Lung related disease: In the Control group, 1 (4%) subject and in the HMUFA group 2 (8%) subjects had a history of lung related disease; one had had pneumonia and two had had asthma.

Liver related disease: In the Control group, 1 (4%) subject and in the HMUFA group 1 (4%) subject had a history of liver related disease; one had had cholecystectomy, and one had had hepatitis.

Other disease: In the Control group, 9 (40%) subjects and in the HMUFA group 9 (40%) subjects had a history of other disease; five had had gout, one had had thyroidectomy, one had had eye operation, two had had pre-eclampsia, one had had arthritis, two had had hiatus hernia, one had had cancer of cervix, one had had GDM, one had had rheumatic fever, and one had peripheral vascular disease.

There were no significant differences in past medical history between the two groups (Table 4.1).

Variable	Control diet	HMUFA diet	Differences P value
-Family history of diabetes % (n)	71% (15)	76% (16)	0.360
-Hypertension % (n) Duration (years)	30% (7) 3.17 ± 1.4	40% (11) 2.98 ± 0.96	0.391 0.912
-Hypercholesterolemia % (n) Duration (years)	24% (6) 0.7 ± 0.4	48% (11) 0.9 ± 0.4	0.079 0.681
-Ischaemic heart disease% (n) Duration (years)	0.08% (2) 3.17 ± 1.4	16% (4) 2.98 ± 0.96	0.392 0.912
Kidney related disease %(n)	12% (3)	12% (3)	1.000
-Liver related disease % (n)	4% (1)	4% (1)	1.000
-Other disease % (n)	40% (9)	40% (9)	1.000

Table 4.1: Past history of medical conditions (on admission). Mean values and SEM are given. P value refers to comparison between the two groups, using t test.

4.1.2 Medication intake (Appendix I, medication intake):

Hypoglycaemic drugs: In the Control group 7 people at the base-line were on one or two types of hypoglycaemic tablets. Two people had withdrawn before 3 months. In HMUFA group 5 people at the base-line were on one or two types of hypoglycaemic tablets. One person withdrew between 3 and 12 months. All participants who remained in the study maintained their medication at the same dose throughout the study. Details are given in Appendix I (medication intake).

Drugs for Hypertension: In the Control group 7 people at the base-line were on one or two types of types of tablets for hypertension treatment. One person withdrew before 3 months. In HMUFA group 5 people at the base-line. One person withdrew between 3 and 12 months. All other participants on medication remained on the same dose, unchanged, throughout the study.

Drugs for Hypercholesterolemia: In the Control group 1 person was on gemfibrozil (Lopid) throughout the study. In HMUFA group 5 people at the base-line and 3 months were on one or two types of tablets for hypercholesterolemia treatment. Between 3 months and 12 months one participant withdrew, and two stopped their medication (one on gemfibrozil, one on simvastin). All other participants on medication remained on the same medication dose, unchanged, throughout the study.

Drugs for heart related problem: In the Control group; 1 subject was taking diltiazem (Cardizem) and isosorbide (Imdure), and in HMUFA group also 1 subject was taking diltiazem (Cardizem) for relief of angina. This medication, and dosage, remained unchanged throughout the study.

Other drugs: The other drugs which were used by participants were: Thyroxine, zyloprim, indocid, zantac, theodur, ventolin, and aspirin. One participant in the Control group took aspirin at 3 months but not at other times in the study, another participant in the Control group ceased taking allopurinol between 3 and 12 months, and one taking thyroxine withdrew from the study before 3 months. In the HMUFA group, two participants using salbutamol inhaler for occasional asthma symptoms had discontinued by 12 months. Otherwise all medication on this category remained unchanged throughout the study.

4.2 Demographic data:

Demographic data taken at baseline are shown in Table 4.2. There were no significant differences between the two experimental groups.

4.3 Anthropometric Measurements:

4.3.1 Base-line:

At Base-line (0 time), there were no significant differences between the groups except: hip, biceps, triceps skinfold thickness, skinfold sum, body density, and body fat % measurements (Figure 4.1 and Table 4.2). The HMUFA group were fatter, but not significantly heavier than the Control group.

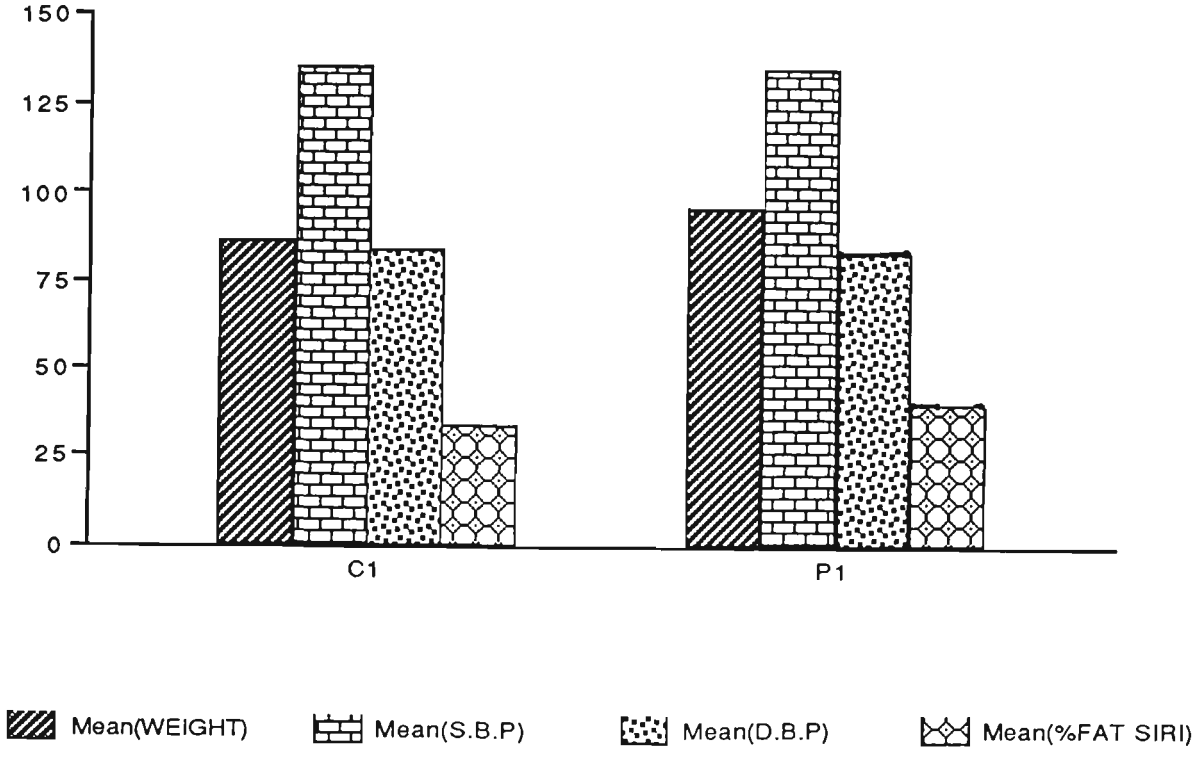

Figure 4.1: Comparison of the mean ± SEM of weight (kg) (P=0.096), SBP (mm Hg) (P=0.968), DBP (mm Hg) (P=0.647), and % body fat (P=0.002), using paired t test, between Control group (C1) and HMUFA group (P1) at base-line.*

Variable	Control diet 0 time	±SEM	HMUFA diet 0 time	±SEM	Differences: P value
No:	24	---	25	---	---
Age	44.8	5	44.6	4	0.739
Sex F/M	9/15	---	14/11	---	---
Wt (Kg)	88.0	3.4	95.8	16	0.096
Height (cm)	169.2	2.2	170.2	11.9	0.741
Systolic B.P (mmHg)	136	3.3	135	15	0.968
Diastolic B.P (mmHg)	85	2.0	83	2.0	0.647
MAP (mmHg)	110	2.4	109.3	2.5	0.752
Pulse	78	2	84	3	0.133
Duration of diabetes (y)	2.2	0.3	3.1	0.9	0.952
Smoker %(n=)	36% (9)	0.1	33% (8)	0.1	0.131
Smoking / day	2.8	2.1	10.0	3.4	0.092
Ex-smoker %(n=)	46% (11)	0.1	43% (10)	0.1	0.688
Ex-smoking / day	11	3.4	12.0	4.0	0.868
Alcohol% (n=)	46% (11)	0.1	52% (13)	0.1	0.674
Alcohol / week	1.4	0.4	2.5	1.2	0.804
Ex-alcohol% (n=)	20% (5)	0.1	12% (3)	0.1	0.417
Ex-alcohol / week	1.3	0.6	0.9	0.5	0.484
Waist (cm)	101.8	2.7	106.7	2.5	0.112
Hip (cm)	110	2.2	115.8	2.0	**0.046***
Waist:hip ratio	0.92	0.01	0.92	0.01	0.872
BMI (Kg.m-2)	30.6	1.0	33.1	0.9	0.072
Biceps (mm)	13.7	1.5	23	2.3	**0.002***
Triceps (mm)	23.3	2.7	30.6	2.2	**0.026***
Subscapular (mm)	28.8	1.9	33.0	1.7	0.109
Suprailiac (mm)	29.7	2.7	35	2.5	0.150
Skinfold sum (mm)	95.5	7.1	121.6	6.2	**0.008***
Body surface area	1.97	0.(	2.06	0.05	0.175
Body Density	1.02	0.003	1.01	0.002	**0.002***
Body fat %	33.8	1.4	39.6	1.0	**0.002***
Exercise:					
Light% (n)	12% (3)	---	8% (2)	---	---
Sedentary% (n)	88% (21)	---	92% (23)	---	---

*Table 4.2: Demographic data and anthropometric measurements at base-line (0 time). Mean values and SEM are given. P value refers to comparison between the two groups, using t test. * shows the significant differences.*

4.3.2 Three months:

At three months time, there was no significant difference between the two groups in demographic data and anthropometric measurements. The measurements of hip, biceps, triceps skinfold thickness, skinfold sum, and body fat % measurements were reduced and body density increased in HMUFA diet and they were not significantly different to Control diet group any more (Table 4.3).

Variable	Control diet 3 months	±SEM	HMUFA diet 3 months	±SEM	Differences: P value
No:	23	---	24	---	---
Sex M / F	8/15	---	14/10	---	---
Wt (Kg)	86.2	3.5	94	16	0.114
Systolic B.P (mmHg)	137	3.6	132	3.7	0.329
Diastolic B.P (mmHg)	85	2.6	79.8	1.7	0.252
MAP (mmHg)	111	3.0	105.7	2.0	0.162
Pulse	74	3.0	79	3.0	0.335
Smoker %(n=)	17% (4)	0.1	33% (8)	0.1	0.220
Smoking / day	3.0	2.2	8.0	2.9	0.169
Alcohol % (n=)	43% (10)	0.1	29% (7)	0.1	0.319
Alcohol / week	4.1	3.0	0.7	0.3	0.215
Waist (cm)	99.9	2.8	101.6	2.7	0.651
Hip (cm)	109.7	2.0	112.9	2.0	0.271
Waist:hip ratio	0.91	0.01	0.90	0.02	0.714
BMI (Kg.m-2)	30.0	1.0	32.5	1.0	0.075
Biceps (mm)	15.9	1.7	19.1	2.3	0.383
Triceps (mm)	22.7	2.0	23.5	1.8	0.503
Subscapular (mm)	31	1.8	29.5	1.4	0.523
Suprailiac (mm)	30.9	2.6	27.5	1.8	0.290
Skinfold sum (mm)	100.5	6.5	99.6	6.0	0.923
Body surface area	1.97	0.05	2.04	0.05	0.283
Body Density	1.02	0.003	1.02	0.002	0.423
Body fat %	35.2	1.4	36.7	1.2	0.402
Exercise:					
Light% (n)	8% (2)	---	8% (2)	---	---
Sedentary% (n)	88% (21)	---	92% (22)	---	---

Table 4.3: Demographic data and anthropometric measurements at 3 months. Mean values and SEM are given. P value refers to comparison between the two groups, using t test.

The HMUFA group had significant reductions in waist (P=0.001*), hip (P=0.002*), biceps (P=0.026*), triceps (P=0.003*), subscapular (P=0.001*), suprailiac

(P=0.001*) skinfold thickness, skinfold sum (P=0.000*), and body fat % measurements (P=0.000*), and significant increases in body density (P=0.000*), when differences between the changes observed at 3 months (compared to base-line) in the two diet groups were compared (Table 4.4, Figure 4.2).

Variable	Control diet 3-0	±SEM	HMUFA diet 3-0	±SEM	Differences: P value
Wt (Kg)	-1.30	0.5	-2.05	0.6	0.360
Systolic B.P (mmHg)	0.65	2.2	-3.96	3.4	0.264
Diastolic B.P (mmHg)	0.65	2.2	-3.96	3.4	0.264
MAP	0.43	1.7	-3.8	2.5	0.168
Pulse / minute	-5.0	3.3	-4.3	3.0	0.859
Smoker (n=)	0.3	0.1	0.3	0.1	1.000
Smoking (/ day)	0.04	0.1	-0.9	0.6	0.292
Alcohol (n=)	-0.04	0.1	-0.2	0.1	0.232
Alcohol / week	2.6	2.8	-1.6	1.2	0.221
Waist (cm)	-1.96	0.6	-5.42	0.8	**0.001***
Hip (cm)	0.2	0.8	-3.3	0.7	**0.002***
Waist:hip ratio	-0.02	0.01	-0.02	0.01	0.970
BMI (Kg.m-2)	-0.42	0.2	-0.73	0.2	0.250
Biceps (mm)	2.5	1.6	-3.8	1.8	**0.026***
Triceps (mm)	0.20	1.8	-7.4	1.8	**0.003***
Subscapular (mm)	2.67	1.6	-4.0	1.0	**0.001***
Suprailiac (mm)	1.69	2.4	-8.4	1.9	**0.001***
Skinfold sum (mm)	1.69	2.4	-8.4	1.9	**0.000***
Body surface area	-0.003	0.01	-0.02	0.01	0.087
Body Density	-0.004	0.002	0.007	0.001	**0.000***
Body fat %	1.71	0.8	-3.15	0.6	**0.000***

*Table 4.4: Comparison of changes observed over the first 3 months of the study in anthropometric and other measurements in subjects on Control diet and those on HMUFA diet. * shows the significant differences between the changes in the two groups.*

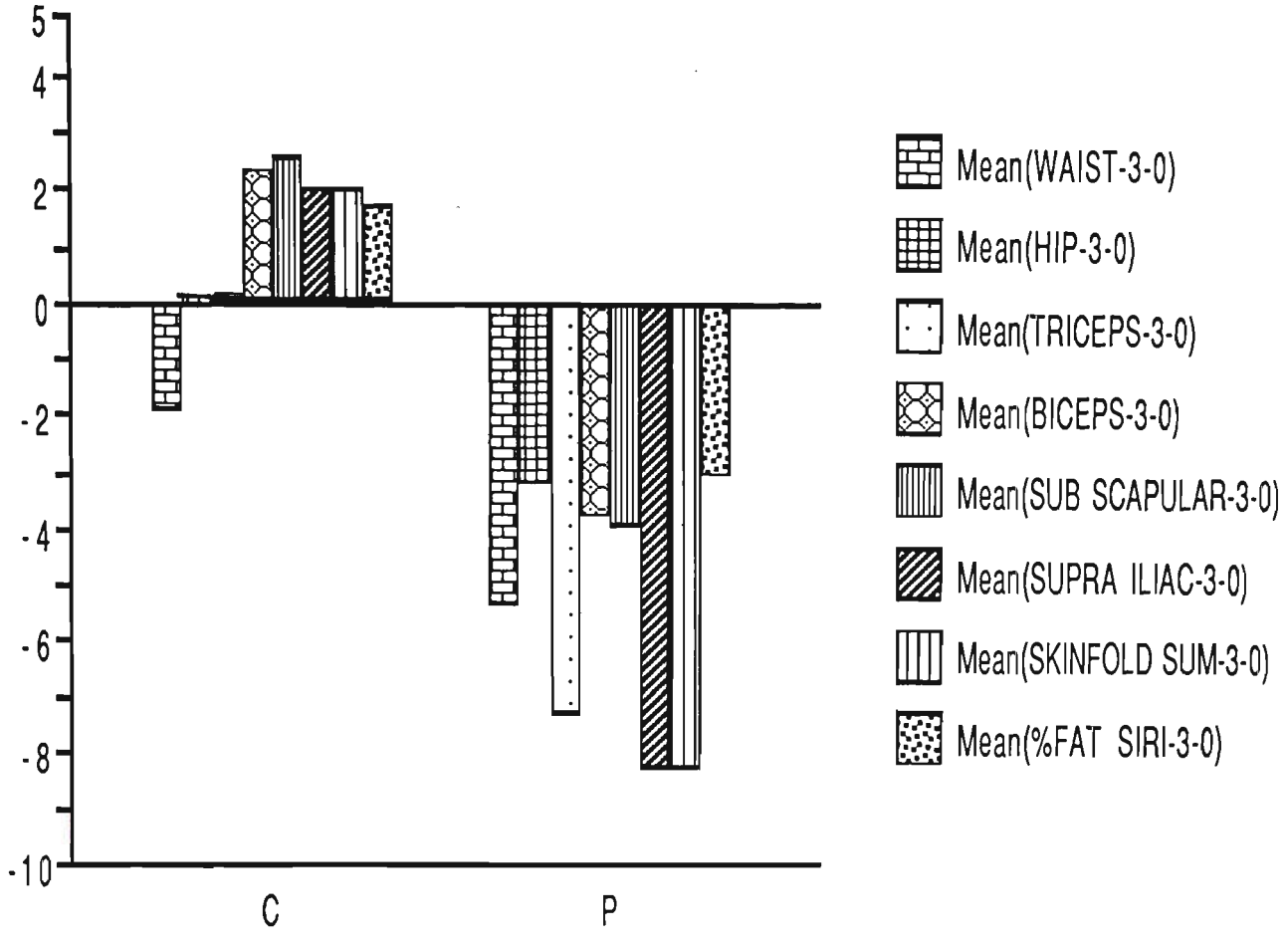

Figure 4.2: Comparison of differences between the changes observed in Control diet and HMUFA diet at 3 months (compared to base-line) in skinfold thickness at waist (P=0.001), hip (P=0.003*), triceps (P=0.003*), biceps (P=0.026*), subscapular (P=0.001*), suprailiac (P=0.001*), skinfold sum (P=0.000*), and body fat % (P=0.000*).*

4.3.3 Twelve months:

When comparing the two groups at twelve months time (Table 4.5 and Figure 4.3 to 4.8), the HMUFA group had higher weight (P=0.044*), hip circumference (P=0.027*), BMI (P=0.012*), and biceps skinfold measurements (P=0.048*) but lower systolic blood pressure (P=0.040*), diastolic blood pressure (P=0.000*), and mean arterial pressure (MAP) (P=0.005*). In relation to alcohol intake although it increased in Control group from 1.4 glass / week at 0 time to 4.1 glass / week at 3

months and 4.4 glass / week at 12 months, compared to HMUFA group which reduced from 1.2 glass / week at 0 time to 0.7 glass / week at 3 months and 0.6 glass / week at 12 months, but there were no significant differences between or within the groups. But the number of individuals who were drinking alcohol was about the same in Control group (11; 0 time-10; 3 months-12; 12 months) but decreased in HMUFA group (13; 0 time-7; 3 months-6; 12 months) and it was not significantly different between the groups. However when the changes at 12 months within the groups (compared to 0 time) were compared there was a significant different between the two groups (P=0.021).

In relation to physical activity there were no significant differences at 0 time, 3 months, or 12 months, between or within the groups.

Variable	Control diet 12 months	±SEM	HMUFA diet 12 months	±SEM	Differences: P value
No:	21		21		
Sex M / F	13 / 8		8 / 13		
Wt (Kg)	86.2	3.9	96.3	3.5	**0.044***
Systolic B.P (mmHg)	136	3.8	127	2.1	**0.040***
Diastolic B.P (mmHg)	84	2.3	73.1	1.4	**0.000***
MAP (mmHg)	110	2.9	100.1	1.7	**0.005***
Pulse / minute	74	2.0	78	3.0	0.354
Smoker% (n=)	19% (4)	0.1	29% (6)	0.1	0.485
Smoking (/ day)	2.8	2.6	6.5	2.7	0.465
Alcohol % (n=)	57% (12)	0.1	29% (6)	0.1	0.067
Alcohol / week	4.4	2.1	0.6	0.3	0.078
Waist (cm)	101.8	3.1	107.4	2.7	0.129
Hip (cm)	110.4	2.1	116.7	1.7	**0.027***
Waist:hip ratio	0.92	0.02	0.92	0.02	0.892
BMI (Kg.m-2)	30.3	1.0	33.6	0.7	**0.012***
Biceps (mm)	15.6	1.2	20	1.8	**0.048***
Triceps (mm)	25.1	2.4	26.5	2.2	0.675
Subscapular (mm)	31	1.7	30.9	1.6	0.906
Suprailiac (mm)	30.9	2.4	30.1	1.8	0.813
Skinfold sum (mm)	102.7	5.9	107.5	6.3	0.580
Body surface area	1.96	0.05	2.07	0.05	0.126
Body Density	1.02	0.003	1.02	0.004	0.810
Body fat %	36.3	1.4	36.8	1.7	0.796
Exercise: Light% (n) Sedentary% (n)	 14% (3) 86% (18)		 5% (1) 95% (20)		

*Table 4.5: Demographic data and anthropometric measurements at 12 months. Mean values and SEM are given. P value refers to comparison between the two groups, using t test. * shows the significant differences (P < 0.05).*

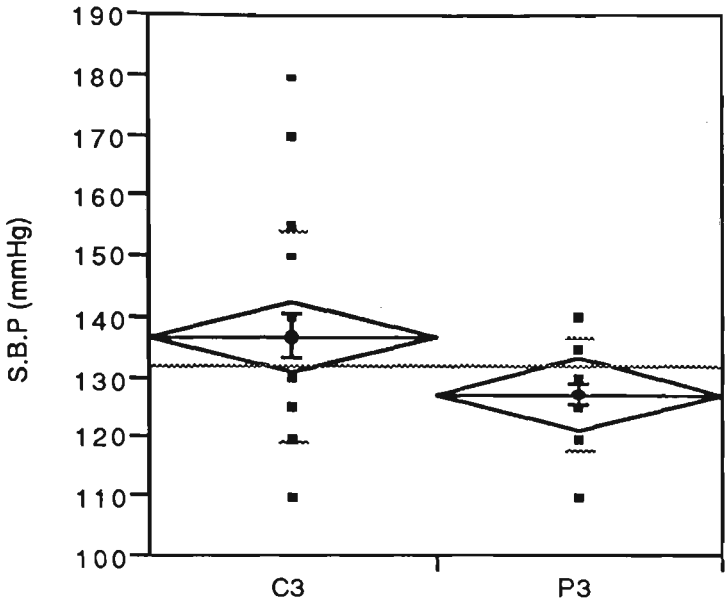

Figure 4.3: Comparison of the mean (± SEM) of systolic blood pressure between Control diet (C3) and HMUFA diet (P3) at 12 months time.
P =0.022 .*

The diamond shows means and 95% confidence limits for each group. The means diamond has a line drawn at the mean (average) value of each diet group. The width of each diamond spans the distance on the horizontal axis proportional to the group size. As it shown in above figure, the Control group (C3) has significant higher mean SBP value than the HMUFA group. The bars represent the standard error of the mean (SEM).

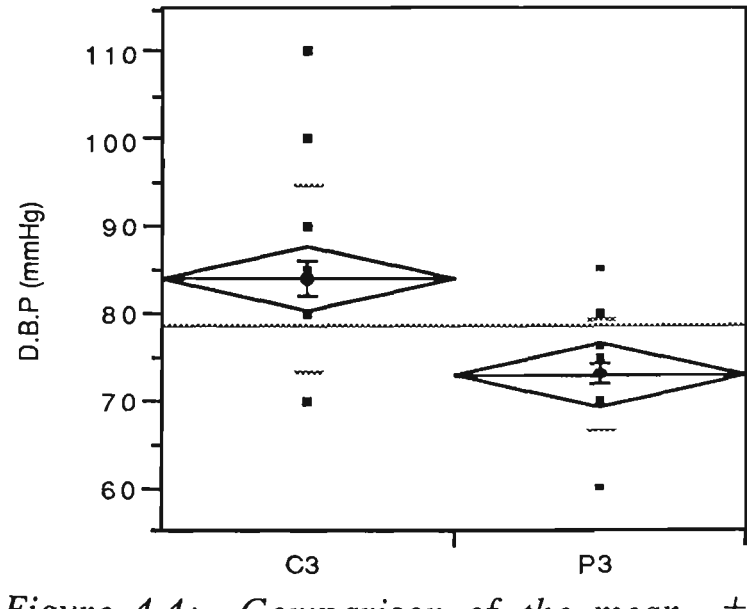

Figure 4.4: Comparison of the mean ± SEM of diastolic blood pressure between Control diet (C3) and HMUFA diet (P3) at 12 months time. P=0.009.*

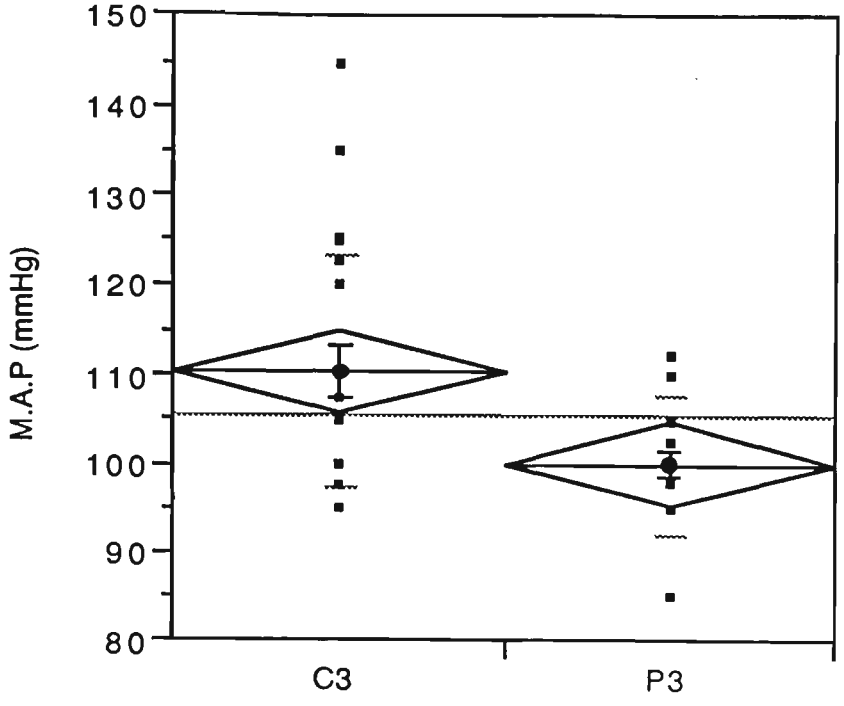

Figure 4.5: Comparison of the mean ± SEM of mean arterial pressure (MAP) between Control diet (C3) and HMUFA diet (P3) at 12 months time. P=0.010.*

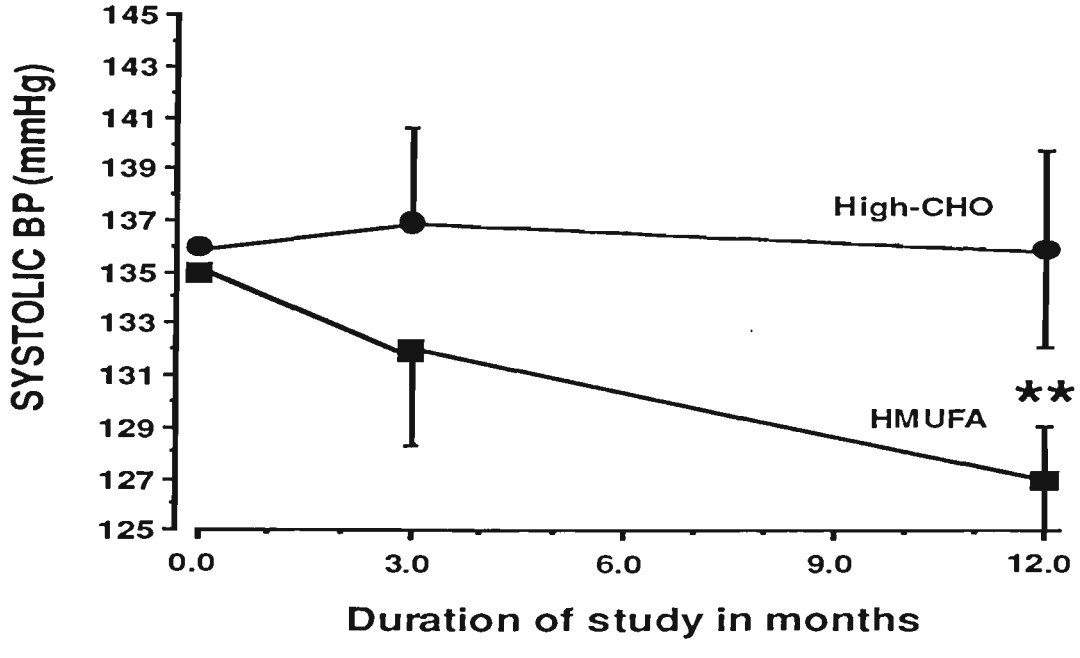

Figure 4.6: The mean ± SEM of systolic blood pressure during one year on Control diet (●) and HMUFA (■) diet (P=0.329 at 3 months; P=0.040 at 12 months). P value refers to comparison between the two groups, using t test.*

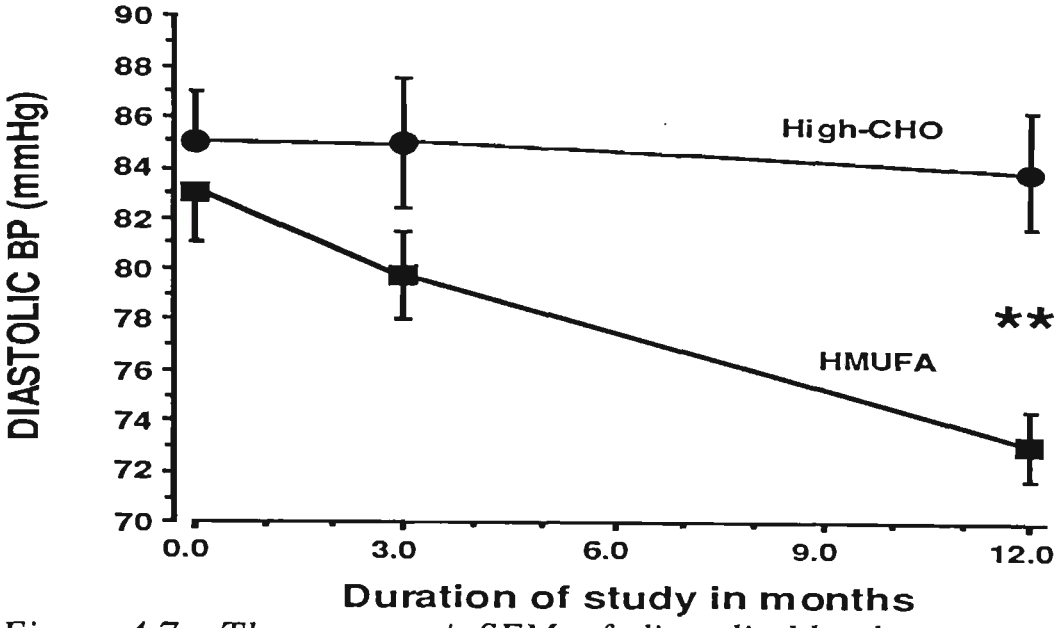

Figure 4.7: The mean ± SEM of diastolic blood pressure during one year on Control diet (●) and HMUFA (■) diet (P=0.252 at 3 months; P=0.000 at 12 months). P value refers to comparison between the two groups, using t test.*

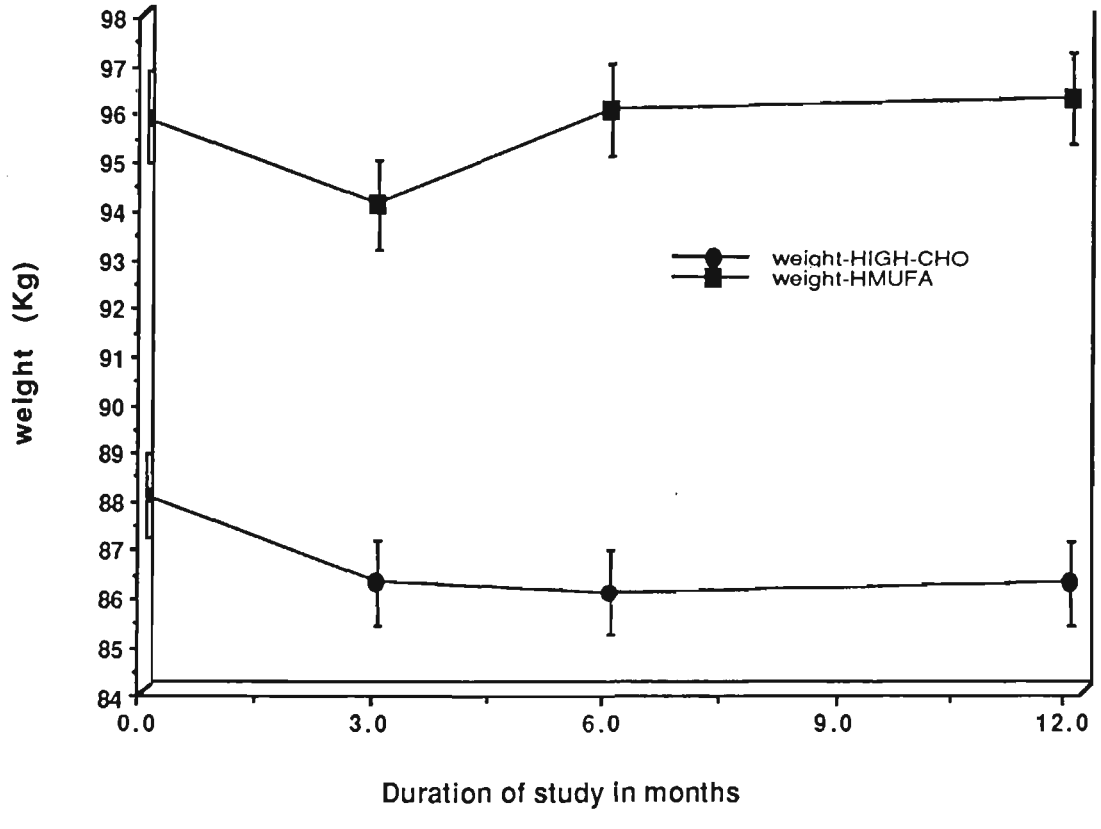

Figure 4.8: The mean ± SEM of weight during one year on Control diet (C) and HMUFA (P) diet. No significant changes had occurred in either group..

Over the 12 months of study the HMUFA group had significant reductions in systolic blood pressure (P=0.022*), diastolic blood pressure (P=0.009*), mean arterial pressure (MAP) (P=0.010*), alcohol intake (P=0.021*), biceps (P=0.034*), triceps (P=0.002*), subscapular (P=0.004*), and suprailiac (P=0.017*) skinfold thickness, skinfold sum (P=0.000*), and body fat % measurements (P=0.000*), and a significant increase in body density (P=0.000*) when compared with the group on the Control diet. Table 4.6 shows these changes over 12 months (12 months value minus baseline value is shown for each dietary group). P values refer to the probability that the observed differences in the changes over 12 months are chance findings. Taken at face value, the data in Table 4.6 suggest that the HMUFA group, over the 12 months of the study, became a little less fat, to a degree which was statistically significant when measured by skinfold thickness but not when measured by weight.

Variable	Control diet 12-0	±SEM	HMUFA diet 12-0	±SEM	Differences: P value
Wt (Kg)	-0.5	0.7	-0.84	1.4	0.805
Systolic B.P (mmHg)	-0.95	2.8	-9.76	3.0	**0.022***
Diastolic B.P (mmHg)	-0.95	2.3	-10.4	2.5	**0.009***
MAP (mmHg)	-0.95	2.3	-10.1	2.6	**0.010***
Pulse / minute	-4.4	2.3	-7.4	1.8	0.322
Smoker% (n=)	0.00	0.1	-0.05	0.1	0.671
Smoking (/ day)	-0.7	0.7	-1.9	1.8	0.779
Alcohol (n=)	0.1	0.1	-0.2	0.1	**0.021***
Alcohol / week	1.4	1.1	-2.0	1.3	0.072
Waist (cm)	0.2	1.1	-1.1	1.4	0.503
Hip (cm)	1.0	0.9	-1.2	1.2	0.149
Waist:hip ratio	-0.01	0.01	-0.002	0.01	0.481
BMI (Kg.m-2)	-0.2	0.3	-0.31	0.5	0.979
Biceps (mm)	2.4	1.1	-3.0	2.1	**0.034***
Triceps (mm)	3.4	1.2	-6.1	2.3	**0.002***
Subscapular (mm)	3.5	1.5	-3.5	1.7	**0.004***
Suprailiac (mm)	1.6	2.4	-7.2	2.6	**0.017***
Skinfold sum(mm)	10.9	4.4	-19.9	6.3	**0.000***
Body surface area	0.01	0.01	-0.003	0.01	0.623
Body Density	-0.006	0.002	0.008	0.002	**0.000***
Body fat %	2.7	1.1	-3.6	1.1	**0.000***

Table 4.6: Comparison of differences in anthropometric and other measurements over 12 months between the groups on the Control diet and HMUFA diet . P values refer to the probability that the observed differences in the changes over 12 months are chance findings

When changes within each group were examined (variables measured at 3 months and 12 months were compared with baseline measurements) it was seen that the HMUFA group had become less fat at 3 months, but this less fat was not maintained to 12 months. Significant reductions were also noted in HMUFA group at 3 months compared with baseline in triceps (P=0.018*), suprailiac (P=0.018*) skinfold, and skinfold sum (P=0.015*) measurements. At 12 months, significant reductions were noted in HMUFA group when comparing within the groups changes in systolic blood pressure (P=0.037*), diastolic blood pressure (P=0.001*), mean arterial pressure (MAP) (P=0.006*). No significant changes was noted within the Control group at 3 months or 12 months (Table 4.7).

	Control (P value)		HMUFA (P value)	
Variable	**3-0**	**12-0**	**3-0**	**12-0**
Wt (Kg)	0.726	0.766	0.681	0.876
Systolic B.P (mmHg)	0.885	0.972	0.355	**↓0.037***
Diastolic B.P (mmHg)	0.996	0.861	0.237	**↓0.001***
MAP (mmHg)	0.929	0.962	0.274	**↓0.006***
Pulse / minute	0.169	0.158	0.153	0.159
Smoker% (n=)	1.000	0.839	0.849	0.602
Smoking (/ day)	0.989	0.999	0.630	0.424
Alcohol (n=)	0.777	0.460	0.108	0.113
Alcohol / week	0.399	0.148	0.154	0.160
Waist (cm)	0.617	0.986	0.170	0.836
Hip (cm)	0.916	0.914	0.319	0.745
Waist:hip ratio	0.413	0.837	0.348	0.926
BMI (Kg.m-2)	0.648	0.818	0.664	0.662
Biceps (mm)	0.346	0.369	0.239	0.326
Triceps (mm)	0.868	0.620	**↓0.018***	0.198
Subscapular(mm)	0.409	0.369	0.124	0.374
Suprailiac (mm)	0.756	0.447	**↓0.018***	0.126
Skinfold sum (mm)	0.611	0.447	**↓0.015***	0.122
Body surface area	0.927	0.862	0.729	0.943
Body Density	0.506	0.241	0.065	0.145
Body fat %	0.498	0.235	0.078	0.156

*Table 4.7: The P value results of comparison with baseline values within each group at 3 months (3-0) and 12 months (12-0) in anthropometric and other measurements. The significant values are shown with *.*

4.4 Lipid and Lipoproteins:

4.4.1 Base-line:

There were no significant differences in lipids or lipoproteins at 0 time, between Control diet and HMUFA diet (Table 4.8).

Variable	Control diet 0 time	±SEM	HMUFA diet 0 time	±SEM	Differences: P value
P. Cholesterol (mmol / L)	5.68	0.3	5.67	0.3	0.757
P.Triglyceride (mmol/L)	2.6	0.4	2.69	0.3	0.347
P. Apo A (g/ L)	1.28	0.07	1.39	0.04	0.882
P. Apo B (g / L)	1.2	0.07	1.21	0.06	0.991
NEFA (mmol / L)	0.39	0.04	0.50	0.04	0.738
VLDL.Cholesterol (mmol / L)	1.12	0.14	0.94	0.1	0.273
VLDL.Triglyceride (mmol / L)	1.21	0.3	1.12	0.2	0.807
VLDL.Apo B (g/L)	0.20	0.02	0.18	0.01	0.338
VLDL.FC (mmol / L)	0.38	0.06	0.32	0.03	0.463
IDL.Cholesterol (mmol / L)	0.11	0.01	0.11	0.01	0.928
IDL.Triglyceride (mmol / L)	0.09	0.01	0.07	0.01	0.624
IDL.Apo B (g/L)	0.05	0.002	0.03	0.001	0.562
IDL.FC (mmol / L)	0.03	0.005	0.03	0.007	0.704
LDL.Cholesterol (mmol / L)	3.40	0.3	3.45	0.2	0.976
HDL.Cholesterol (mmol / L)	1.07	0.1	0.99	0.05	0.912
LDL:HDL:R	3.7	0.3	3.8	0.4	0.711
HDL.Apo A (g / L)	1.26	0.08	1.16	0.05	0.453
CETP (%)	36	1.2	37.5	1.8	0.532

Table 4.8: Lipid and lipoproteins results at base-line (0 time). Mean values and SEM are given. P value refers to comparison between the two groups, using t test.

4.4.2 Three months:

There were no significant changes between the two groups at 3 months time (Table 4.9).

Variable	Control diet 3 months	±SEM	HMUFA diet 3 months	±SEM	Differences: P value
P. Cholesterol (mmol / L)	5.41	0.3	5.38	0.2	0.663
P. Triglyceride (mmol / L)	2.6	0.4	2.58	0.3	0.865
P. Apo A (g / L)	1.40	0.06	1.39	0.04	0.882
P. Apo B (g / L)	1.15	0.06	1.15	0.04	0.991
NEFA (mmol / L)	0.45	0.03	0.44	0.04	0.738
VLDL.Cholesterol (mmol / L)	1.21	0.1	1.08	0.1	0.273
VLDL.Triglyceride (mmol / L)	1.30	0.2	1.27	0.16	0.807
VLDL.Apo B (g / L)	0.22	0.02	0.20	0.01	0.338
VLDL.FC (mmol / L)	0.35	0.03	0.32	0.03	0.463
IDL.Cholesterol (mmol / L)	0.11	0.01	0.12	0.02	0.617
IDL.Triglyceride (mmol / L)	0.07	0.01	0.06	0.01	0.992
IDL.Apo B (g / L)	0.03	0.001	0.03	0.001	0.371
IDL.FC (mmol / L)	0.02	0.004	0.02	0.003	0.941
LDL.Cholesterol (mmol / L)	3.0	0.2	3.16	0.2	0.522
HDL.Cholesterol (mmol / L)	1.09	0.07	1.04	0.03	0.718
LDL:HDL:R	2.9	0.2	3.1	0.1	0.492
HDL.Apo A(g / L)	1.38	0.05	1.30	0.05	0.217
CETP (%)	33	1.7	34	1.7	0.532

Table 4.9: Lipid and lipoproteins results at 3 months. Mean values and SEM are given. P value refers to comparison between the two groups, using t test.

But there was significant difference between the changes observed at 3 months (compared to base-line), in NEFA (P=0.022*), comparing the two diet group (Table 4.10).

Variable	Control diet 3-0	±SEM	HMUFA diet 3-0	±SEM	Differences: P value
P. Cholesterol (mmol / L)	-0.31	0.2	-0.11	0.2	0.503
P. Triglyceride (mmol / L)	0.29	0.3	-0.07	0.2	0.202
P. Apo A (g / L)	0.09	0.05	-0.02	0.05	0.149
P. Apo B (g / L)	-0.07	0.04	-0.03	0.03	0.540
NEFA (mmol / L)	0.06	0.05	-0.07	0.05	**0.022***
VLDL.Cholesterol (mmol / L)	0.06	0.1	0.15	0.06	0.547
VLDL.Triglyceride (mmol / L)	0.06	0.3	0.18	0.1	0.856
VLDL.Apo B (g / L)	0.01	0.02	0.03	0.01	0.614
VLDL.FC (mmol / L)	-0.03	0.05	0.003	0.02	1.000
IDL.Cholesterol (mmol / L)	-0.005	0.01	-0.003	0.03	0.573
IDL.Triglyceride (mmol / L)	-0.02	0.01	0.03	0.01	0.632
IDL.Apo B (g / L)	-0.02	0.02	0.001	0.002	0.898
IDL.FC (mmol / L)	-0.01	0.006	-0.02	0.008	0.798
LDL.Cholesterol (mmol / L)	-0.49	0.2	-0.14	0.1	0.209
HDL.Cholesterol (mmol / L)	0.045	0.1	0.05	0.1	0.766
LDL:HDL:R	-0.89	0.4	-0.61	0.4	0.292
HDL.Apo A (g / L)	0.15	0.05	0.15	0.06	0.683
CETP (%)	-3.9	1.0	-2.9	1.2	0.826

Table 4.10: Comparison of differences in lipid and lipoprotein between the changes observed in Control diet and HMUFA diet at 3 months (compared to base-line).

4.4.3 Twelve months:

At twelve months time (Figures 4.9 to 4.19), there were significant reductions in: NEFA (P=0.000*), VLDL-cholesterol (P=0.010*), VLDL-TG (P=0.015*), VLDL-FC (P=0.033*), CETPA% (P=0.000*), and significant increases in HDL-C (P=0.001*), and HDL-Apo A (P=0.003*), in the group on the HMUFA diet, when compared with the group on the Control diet (Table 4.11).

Variable	Control diet 12 months	±SEM	HMUFA diet 12 months	±SEM	Differences: P value
P. Cholesterol (mmol / L)	5.09	0.2	5.06	0.2	0.93
P. Triglyceride (mmol / L)	2.7	0.4	2.24	0.2	0.429
P. Apo A (g / L)	1.30	0.05	1.33	0.05	0.647
P. Apo B (g / L)	1.12	0.06	1.14	0.06	0.907
NEFA (mmol/l)	0.86	0.09	0.41	0.03	**0.000***
VLDL.Cholesterol (mmol/l)	1.1	0.1	0.72	0.1	**0.010***
VLDL.Triglyceride (mmol / L)	1.76	0.3	0.84	0.1	**0.015***
VLDL.Apo B (g / L)	0.21	0.02	0.16	0.02	0.096
VLDL.FC (mmol/l)	0.40	0.06	0.25	0.03	**0.033***
(mmol/l)	0.10	0.01	0.06	0.01	0.080
IDL.Triglyceride (mmol/l)	0.07	0.01	0.05	0.01	0.524
IDL.Apo B (g / L)	0.03	0.002	0.03	0.002	0.632
IDL.FC (mmol / L)	0.03	0.007	0.015	0.004	0.184
LDL.Cholesterol (mmol / L)	3.1	0.3	2.97	0.2	0.722
HDL.Cholesterol (mmol/l)	0.78	0.06	1.06	0.05	**0.001***
LDL:HDL:R	5.5	1.3	2.9	0.2	0.055
HDL.Apo A (g / L)	1.12	0.06	1.34	0.05	**0.003***
CETP (%)	54	1.3	31	2.2	**0.000***

Table 4.11: Lipid and lipoprotein results at 12 months. Mean values and SEM are given. P value refers to comparison between the two groups, using t test.

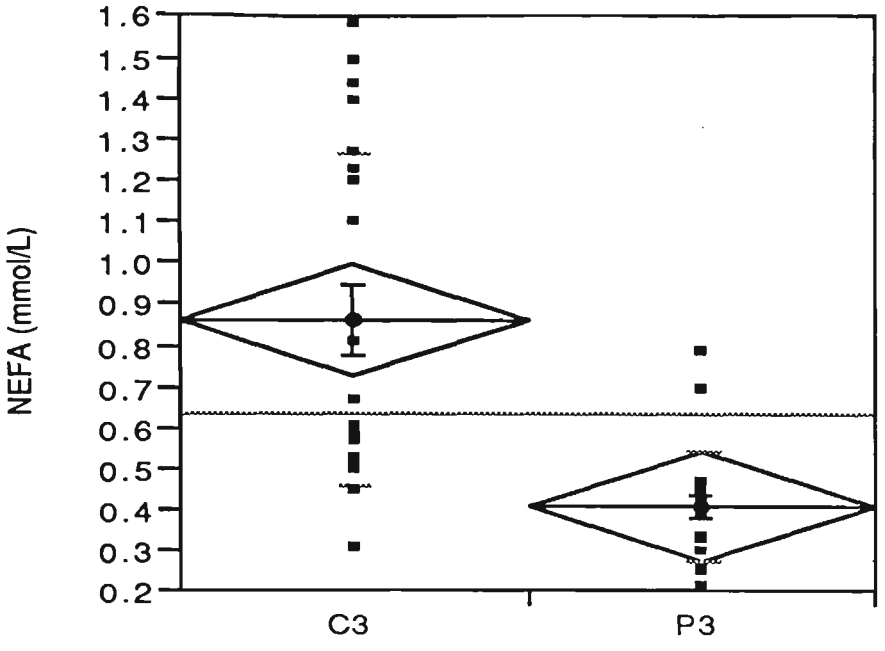

Figure 4.9: Comparison of the mean ± SEM of NEFA between Control diet (C3) and HMUFA diet (P3) at 12 months time. (P=0.000)*

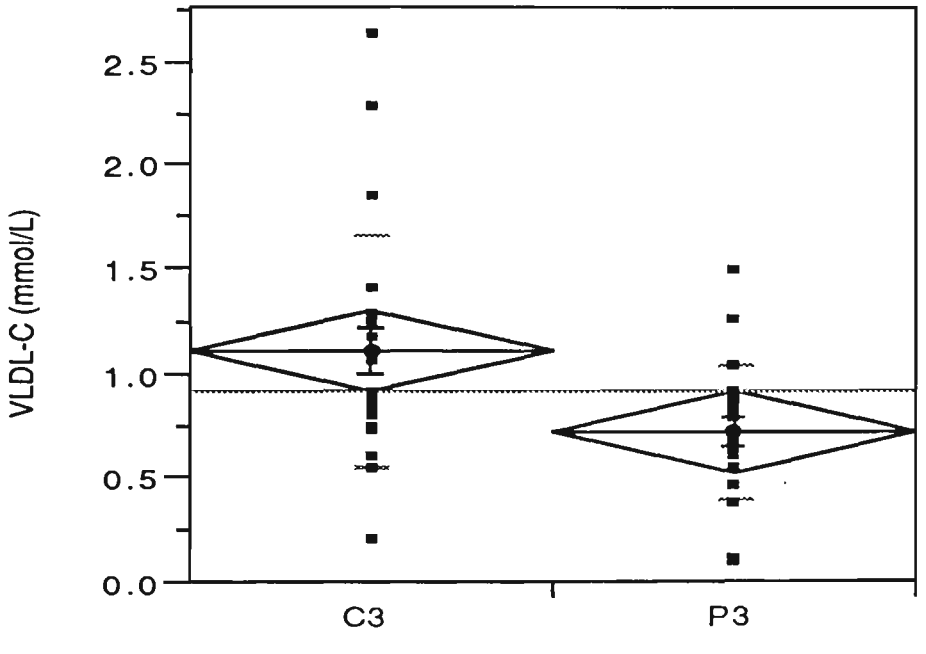

Figure 4.10: Comparison of the mean ± SEM of VLDL-C between Control diet (C3) and HMUFA diet (P3) at 12 months time. (P=0.010)*

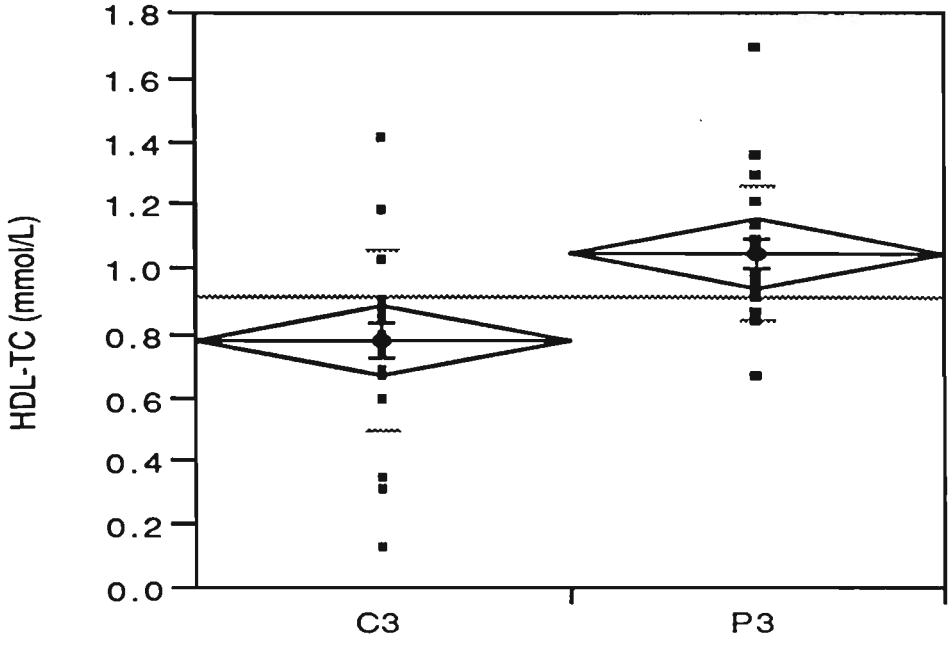

Figure 4.11: Comparison of the mean ± SEM of HDL-C between Control diet (C3) and HMUFA diet (P3) at 12 months time. (P=0.001)*

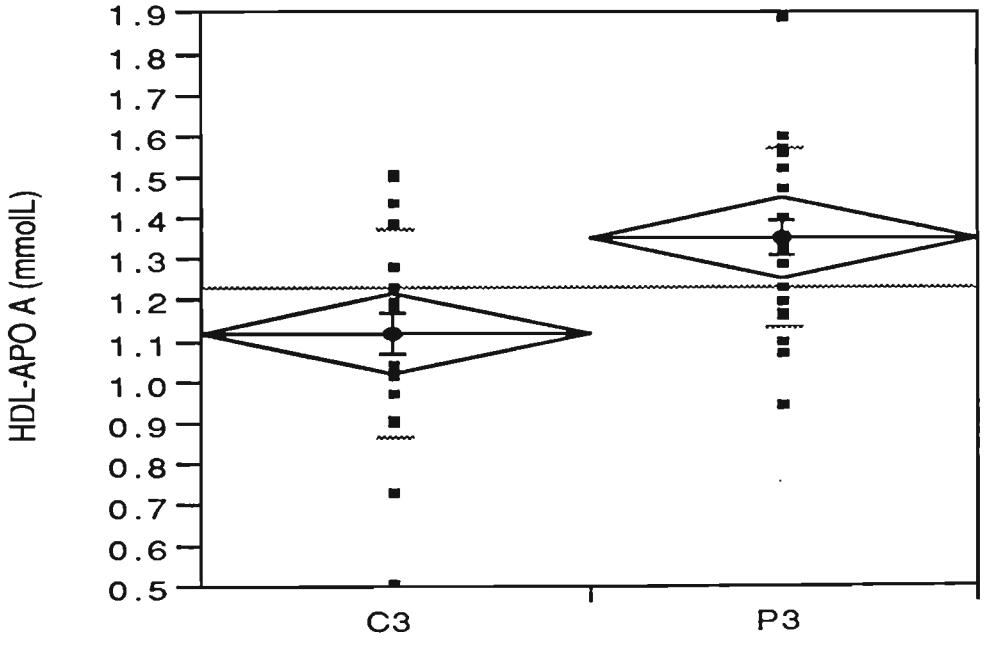

Figure 4.12: Comparison of the mean ± SEM of HDL-Apo A between Control diet (C3) and HMUFA diet (P3) at 12 months time. (P=0.010)*

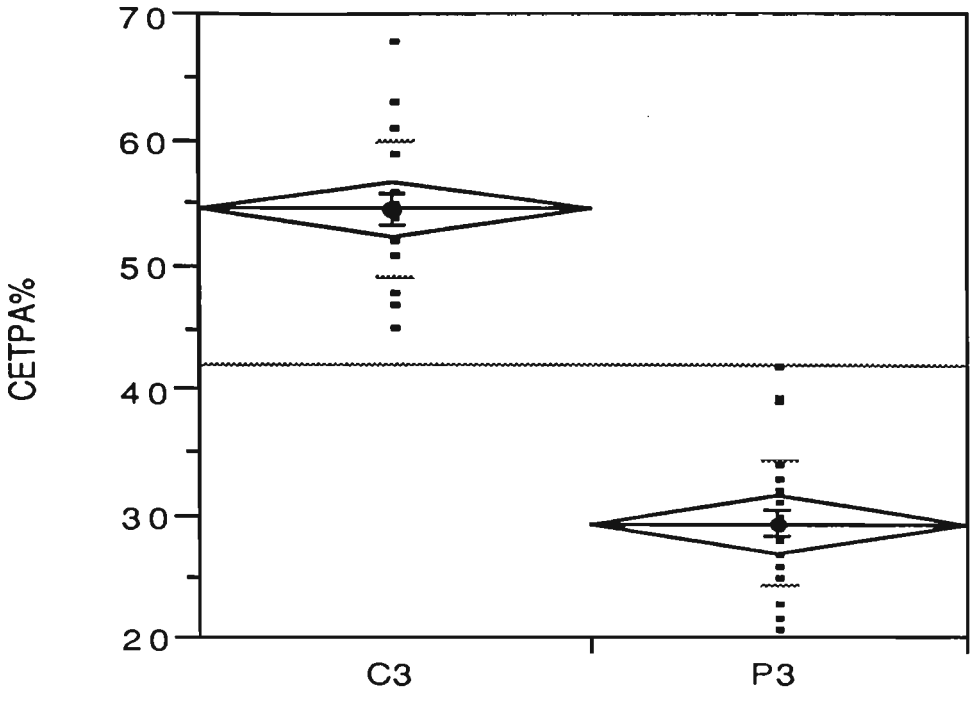

Figure 4.13: Comparison of the mean ± SEM of CETP activity (%) between Control diet (C3) and HMUFA diet (P3) at 12 months time. (P=0.000)*

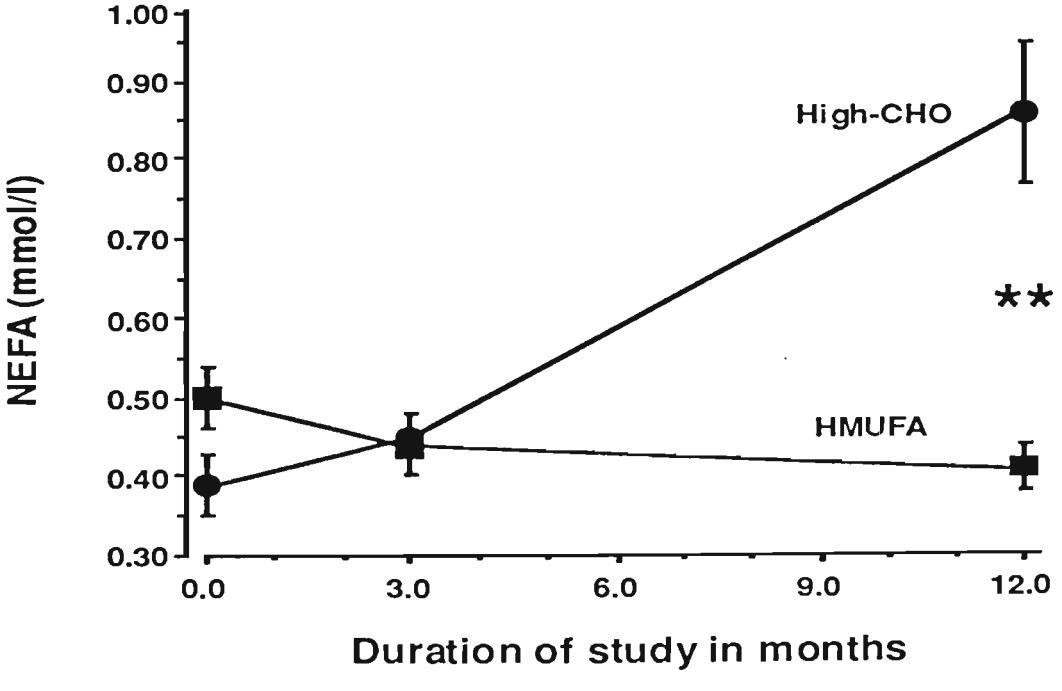

Figure 4.14: Mean of NEFA during 12 months in Control diet (●) and HMUFA (■) diet. P value refers to comparison between the two groups, using t test.

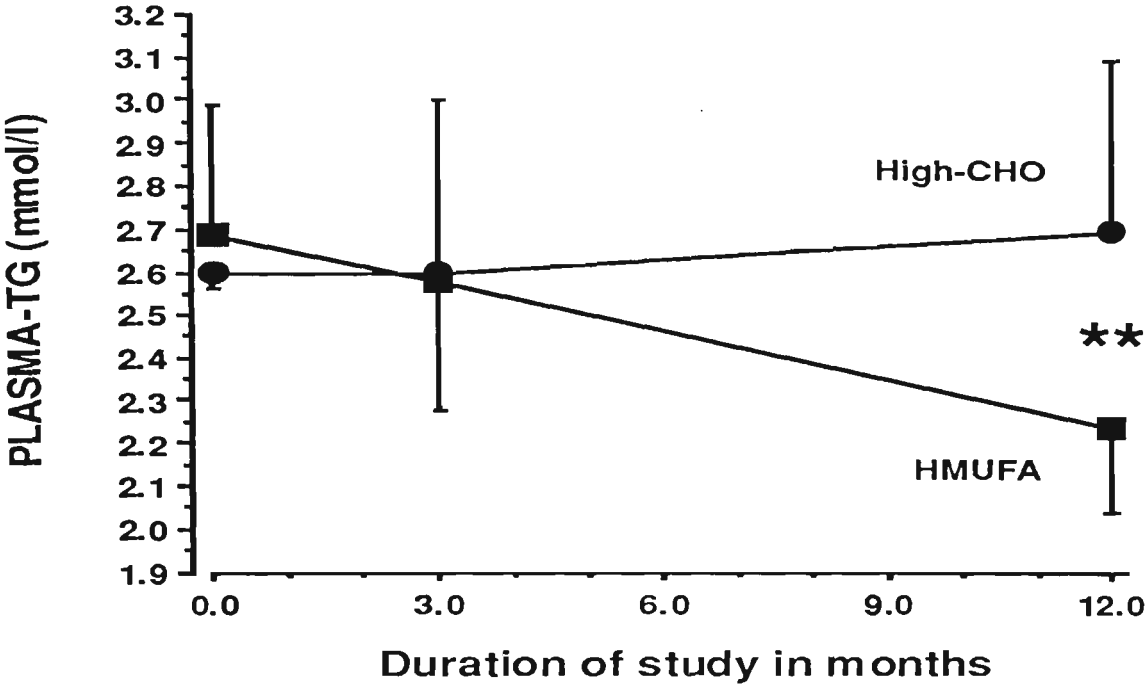

Figure 4.15: Mean of plasma triglyceride during 12 months in Control diet (●) and HMUFA (■) diet. P value refers to comparison between the two groups, using t test.

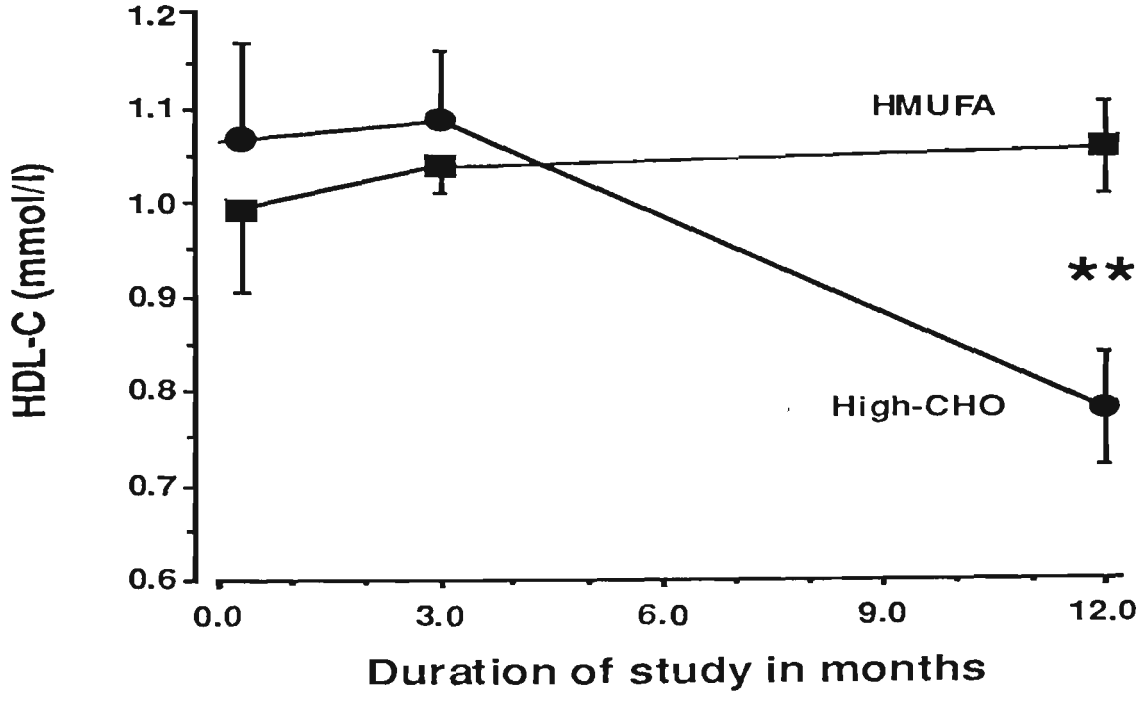

Figure 4.16: The mean ± SEM of HDL cholesterol during 12 months in Control diet (●) and HMUFA (■) diet. P value refers to comparison between the two groups, using t test.

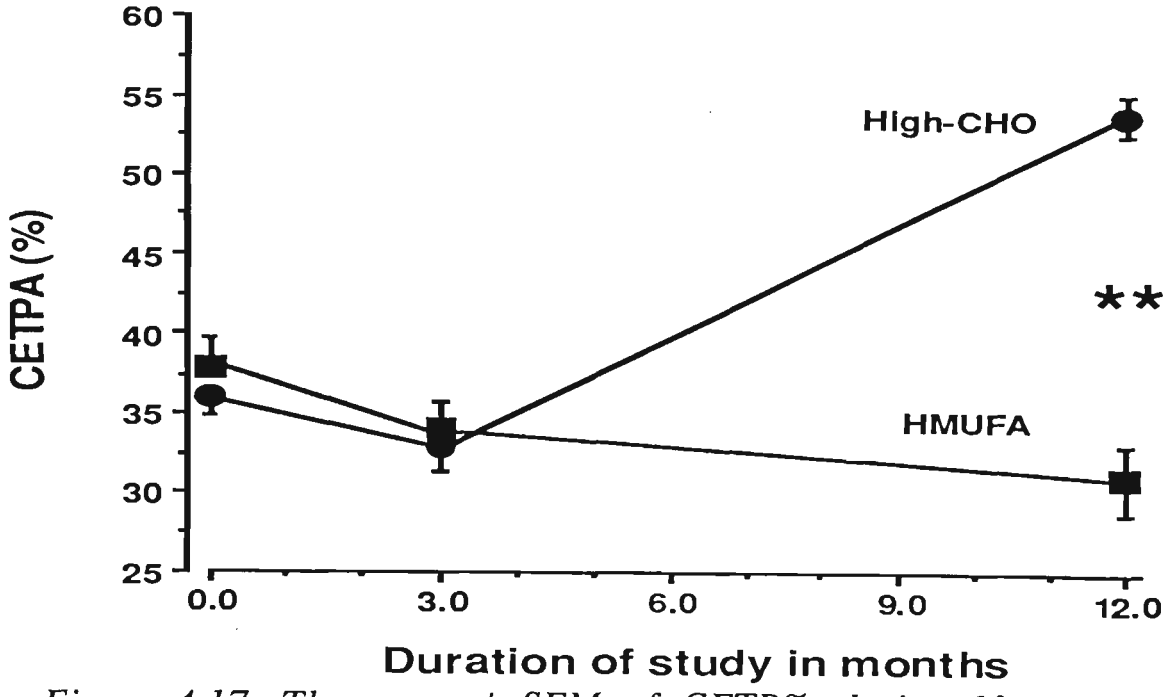

Figure 4.17: The mean ± SEM of CETP% during 12 months in Control diet (●) and HMUFA (■) diet. P value refers to comparison between the two groups, using t test.

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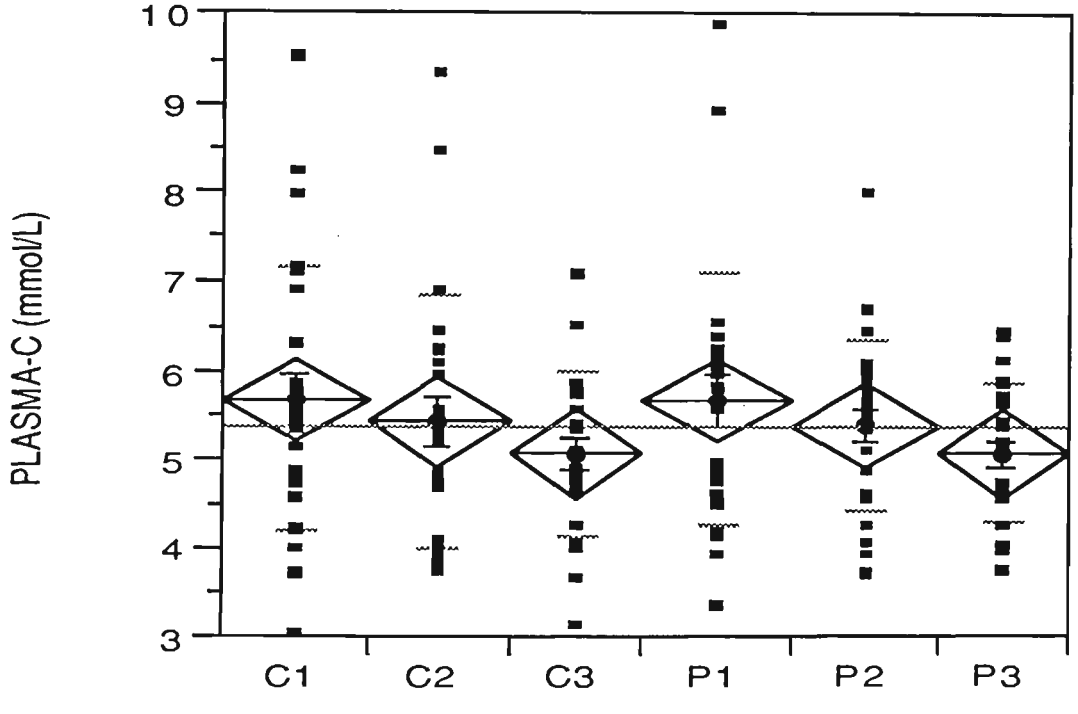

Figure 4.18: The mean ± SEM of Plasma cholesterol during 12 months in Control diet (C1-C2- C3) and HMUFA diet (P1-P2-P3). C1&P1= 0 time, C2&P2= 3 months, C3&P3= 12 months.

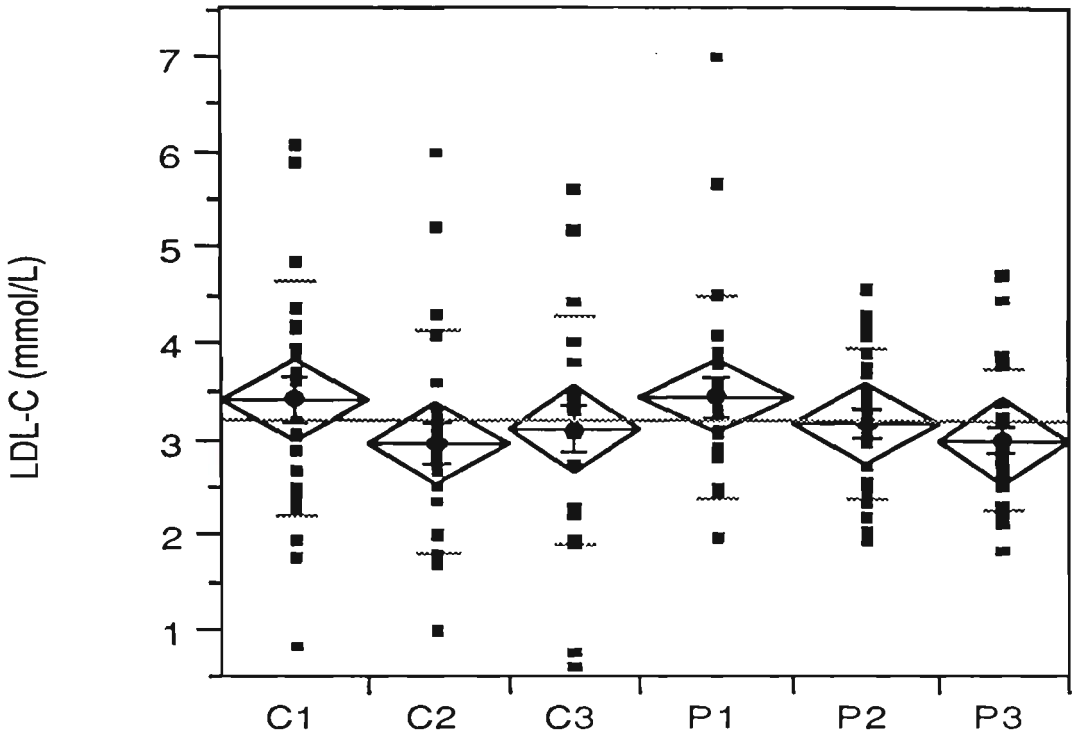

Figure 4.19: The mean ± SEM of LDL-cholesterol during 12 months in Control diet (C1-C2- C3) and HMUFA diet (P1-P2-P3). C1&P1= 0 time, C2&P2= 3 months, C3&P3= 12 months.

There were also significant differences between the changes observed at 12 months (compared to base-line), in plasma-TG (P=0.050*), NEFA (P=0.000*), VLDL-TG (P=0.011*), IDL-C (P=0.019*), IDL-FC (P=0.029*), HDL-C (P=0.005*), HDL-Apo A (P=0.006*), and CETPA% (P=0.000*), comparing the two diet group (Table 4.12 and Figure 4.20).

Variable	Control diet 12-0	±SEM	HMUFA diet 12-0	±SEM	Differences: P value
P. Cholesterol (mmol / L)	-0.45	0.3	-0.43	0.2	0.949
P. Triglyceride (mmol / L)	0.43	0.3	-0.40	0.3	**0.050***
P. Apo A (g / L)	0.02	0.06	-0.08	0.06	0.205
P. Apo B (g / L)	-0.06	0.08	-0.04	0.05	0.949
NEFA (mmol / L)	0.48	0.08	-0.1	0.04	**0.000***
VLDL.Cholesterol (mmol / L)	0.07	0.1	-0.21	0.1	0.279
VLDL.Triglyceride (mmol / L)	0.73	0.3	-0.27	0.2	**0.011***
VLDL.Apo B (g / L)	0.01	0.02	-0.02	0.02	0.291
VLDL.FC (mmol / L)	0.07	0.06	-0.07	0.05	0.072
IDL.Cholesterol (mmol / L)	-0.01	0.01	-0.06	0.01	**0.019***
IDL.Triglyceride (mmol / L)	-0.01	0.01	-0.05	0.02	0.087
IDL.Apo B (g / L)	0.004	0.002	0.002	0.003	0.507
IDL.FC (mmol / L)	-0.007	0.006	-0.03	0.009	**0.029***
LDL.Cholesterol (mmol / L)	-0.33	0.3	-0.30	0.2	0.936
HDL.Cholesterol (mmol / L)	-0.29	0.1	0.05	0.05	**0.005***
LDL:HDL:R	1.8	1.3	-0.7	0.5	0.182
HDL.Apo A (g / L)	-0.08	0.07	0.19	0.06	**0.006***
CETP (%)	18.8	2.0	-5.6	2.4	**0.000***

Table 4.12: Comparison of differences in lipid and lipoprotein between the changes observed in Control diet and HMUFA diet at 12 months (compared to base-line).

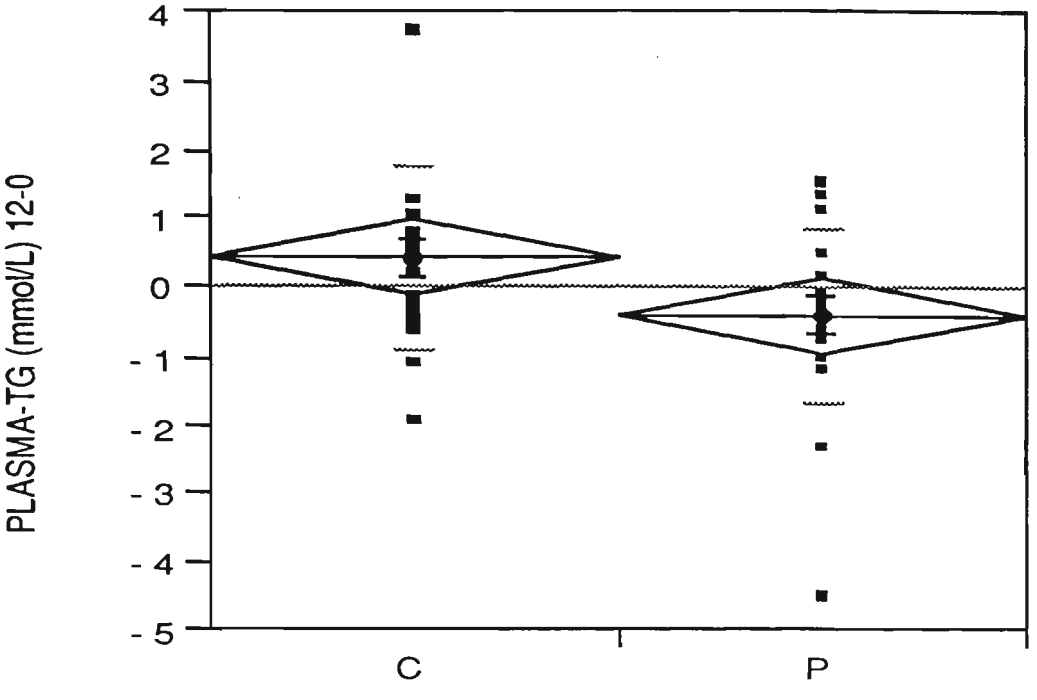

Figure 4.20: Comparison of differences in plasma-TG between the changes observed in Control diet and HMUFA diet at 12 months (compared to base-line) P=0.000.*

There was significant reduction in IDL-TG (P=0.043*) and IDL-FC (P=0.023*) in comparing the changes within HMUFA group at 3 months. No significant changes were noted within the Control group at 3 months.(Table 4.13).

There was also significant reduction in VLDL-C (P=0.039*), IDL-C (P=0.001*), IDL-TG (P=0.009*), IDL-FC (P=0.007*), and CETPA% (P=0.029*) and significant increase in HDL-Apo A (P=0.016*), in comparing the changes within HMUFA group at 12 months. In Control group there was significant increase in NEFA (P=0.000*), and CETPA% (P=0.000*), and significant decrease in HDL-C (P=0.019*), in comparing the changes within the group at 12 months (Table 4.13).

	Control (P value)		HMUFA (P value)	
Variable	3-0	12-0	3-0	12-0
P. Cholesterol (mmol / L)	0.534	0.129	0.408	0.085
P. Triglyceride (mmol / L)	0.629	0.928	0.785	0.271
P. Apo A (g / L)	0.253	0.814	0.737	0.386
P. Apo B (g / L)	0.527	0.391	0.412	0.419
NEFA (mmol / L)	0.263	↑0.000*	0.270	0.088
VLDL.Cholesterol (mmol / L)	0.619	0.911	0.229	↓0.039*
VLDL.Triglyceride (mmol / L)	0.766	0.197	0.535	0.150
VLDL.Apo B (g / L)	0.451	0.970	0.173	0.314
VLDL.FC (mmol / L)	0.724	0.779	0.93	0.083
IDL.Cholesterol (mmol / L)	0.923	0.554	0.911	↓0.001*
IDL.Triglyceride (mmol / L)	0.359	0.401	↓0.043	↓0.009*
IDL.Apo B (g / L)	0.413	0.475	0.521	0.261
IDL.FC (mmol / L)	0.116	0.453	↓0.023*	↓0.007*
LDL.Cholesterol (mmol / L)	0.222	0.390	0.296	0.097
HDL.Cholesterol (mmol / L)	0.839	↓0.019*	0.474	0.357
LDL:HDL:R	0.051	0.153	0.085	0.093
HDL.Apo A(g / L)	0.177	0.159	0.060	↑0.016*
CETP (%)	0.148	↑0.000*	0.165	↓ 0.029*

*Table 4.13: The P value results of comparison within the group at 3 months (3-0) and 12 months (12-0) in lipid and lipoprotein. The significant values are shown with *.*

4.5 Glucose and indices of glucose metabolism:

4.5.1 Base-line:

At 0 time, there were no significant differences in: fasting plasma glucose, Hb A1c, insulin, insulin:FBG ratio, or C-peptide between the two diet groups (Table 4.14).

Variable	Control diet 0 time	±SEM	HMUFA diet 0 time	±SEM	Differences: P value
Fasting plasma glucose mmol/L	8.5	0.6	9.2	0.6	0.317
Hb A1c %	7.8	0.5	8.5	0.4	0.066
Insulin mIU/L	10.3	1.2	12.8	1.9	0.516
Insulin:FBG:R	1.3	0.2	1.5	0.2	0.562
C-peptide ug/L	3.8	0.3	4.3	0.3	0.270

Table 4.14: Glucose and indices of glucose metabolism at base-line (0 time). Mean values and SEM are given. P value refers to comparison between the two groups, using t test.

4.5.2 Three months:

There were no significant differences between the Control diet and HMUFA diet at three months time (Table 4.15).

Variable	Control diet 3 months	±SEM	HMUFA diet 3 months	±SEM	Differences: P value
Fasting plasma glucose (mmol/L)	8.6	0.7	8.6	0.6	0.678
Hb A1c %	7.8	0.5	8.2	0.4	0.163
Insulin mIU/L	10.3	1.0	14.7	2.4	0.551
Insulin:FBG:R	1.3	0.2	1.7	0.2	0.437
C-peptide ug/L	4.0	0.3	4.48	0.4	0.655

Table 4.15: Glucose and indices of glucose metabolism at 3 months. Mean values and SEM are given. P value refers to comparison between the two groups, using t test.

There were no significant differences between the changes observed at 3 months (compared to base-line) in the two diet groups (Table 4.16).

Variable	Control diet 3-0	±SEM	HMUFA diet 3-0	±SEM	Differences: P value
Fasting plasma glucose (mmol/L)	0.04	0.5	-0.6	0.4	0.544
Hb A1c %	-0.05	0.2	-0.33	0.3	0.609
Insulin mIU/L	-0.2	0.9	1.51	1.6	0.990
Insulin:FBG:R	0.01	0.1	0.24	0.2	0.292
C-peptide ug/L	0.16	0.2	0.1	0.2	0.856

Table 4.16: Comparison of differences in glucose and indices of glucose metabolism between the changes observed in Control diet and HMUFA diet at 3 months (compared to base-line).

4.5.3 Twelve months:

There was a significant increase in insulin:FBG ratio in those on the HMUFA diet. No other significant changes was noted in comparing the two diet groups at 12 months (Table 4.17).

Variable	Control diet 12 months	±SEM	HMUFA diet 12 months	±SEM	Differences: P value
Fasting plasma glucose (mmol/L)	9.5	0.8	8.3	0.6	0.225
Hb A1c %	8.0	0.5	7.9	0.4	0.983
Insulin mIU/L	12.0	1.1	15.7	2.0	0.121
Insulin:FBG:R	1.4	0.1	1.9	0.2	**0.033***
C-peptide ug/L	3.7	0.3	8.3	0.6	0.114

Table 4.17: Glucose and indices of glucose metabolism at 12 months. Mean values and SEM are given. P value refers to comparison between the two groups, using t test.

There was a significant difference between the changes observed at 12 months (compared to base-line), in FBG (P=0.026*); mean fasting plasma glucose rose 1.0 mmol/L in those on the Control diet during 12 months, but fell 0.44 mmol/L in those on the HMUFA diet in the same time period (Table 4.18 and Figures 4.21-4.22).

Variable	Control diet 12-0	±SEM	HMUFA diet 12-0	±SEM	Differences: P value
Fasting plasma glucose (mmol/L)	1.0	0.5	-0.44	0.3	**0.026***
Hb A1c %	0.1	0.3	-0.2	0.3	0.217
Insulin mIU/L	2.0	1.0	1.6	1.6	0.990
Insulin:FBG:R	0.13	0.1	0.32	0.2	0.381
C-peptide ug/L	-0.12	0.2	-0.2	0.3	0.791

Table 4.18: Comparison of differences in glucose and indices of glucose metabolism between the changes observed in Control diet and HMUFA diet at 12 months (compared to base-line).

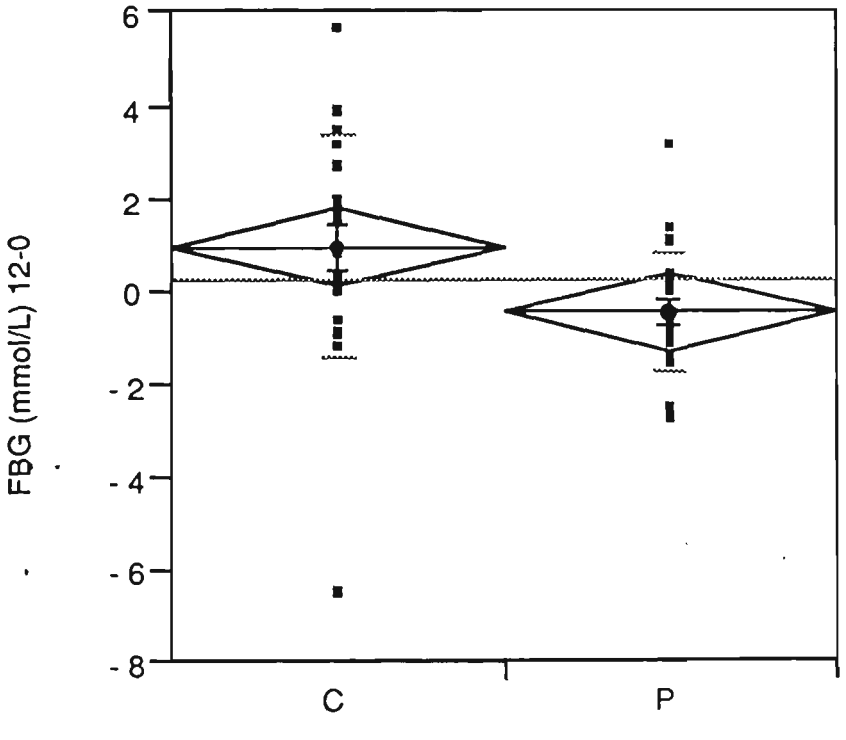

Figure 4.21: Comparison of differences in fasting plasma glucose (FBG) between the changes observed in Control diet and HMUFA diet at 12 months (compared to base-line) P=0.026.*

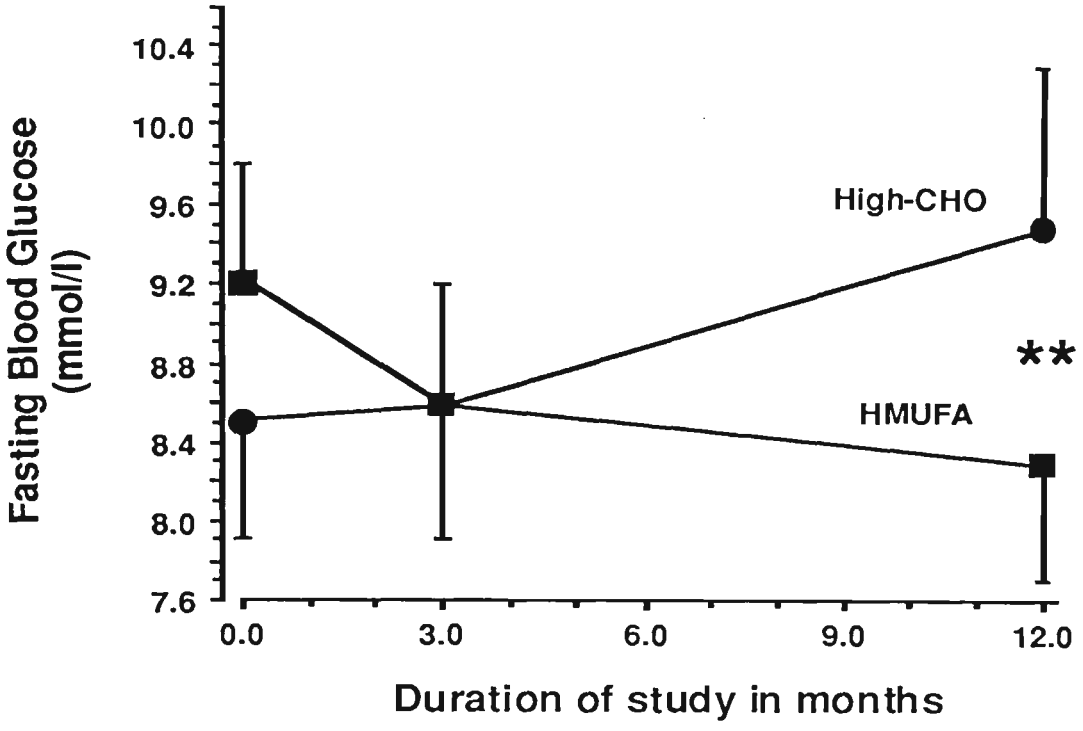

*FIGURE 4.22: The mean ± SEM of fasting plasma glucose during 12 months in Control diet (●) and HMUFA (■) diet. P value refers to comparison between the two groups, using t test. ** indicates the significant differences between the changes occurred at 12 months (P=0.026)*

There were not any significant changes at 3 months or 12 months within the groups (when compared with the baseline) (Table 4.19).

	Control		HMUFA	
Variable	**3-0**	**12-0**	**3-0**	**12-0**
Fasting plasma glucose (mmol/L)	0.903	0.321	0.526	0.306
Hb A1c %	0.997	0.859	0.479	0.293
Insulin mIU/L	0.997	0.329	0.548	0.302
Insulin:FBG:R	0.881	0.661	0.383	0.059
C-peptide ug/L	0.672	0.679	0.787	0.997

Table 4.19: The P value results of comparison within the group at 3 months (3-0) and 12 months (12-0) in glucose and indices of glucose metabolism.

4.6 Euglycemic clamp study:

4.6.1 Base-line:

At 0 time (Base-line), there were no significant differences in glucose uptake, prime infusion of insulin, or total insulin infused between the Control group and HMUFA group (Table 4.24).

Variable	Control diet 0 time	±SEM	HMUFA diet 0 time	±SEM	Differences: P value
No of patients	11		16		
Glucose uptake $mg.m^{-2}.min^{-1}$	130	20	111	15	0.443
Total Insulin infused (10 min. prime) $mU.m^{-2}.min^{-1}$	16	0.4	17	0.7	0.195
Total Insulin infused (total, without the prime) $mU.m^{-2}.min^{-1}$	164	20	176	10	0.348

Table 4.20: Euglycemic clamp study results at base-line (0 time). Mean values and SEM are given. P value refers to comparison between the two groups, using t test.

4.6.2 Three months time:

At 3 months time, there were no significant differences in glucose uptake, or total insulin infused, between the Control group and HMUFA group (Table 4.21).

Variable	Control diet 3 months	±SEM	HMUFA diet 3 months	±SEM	Differences: P value
No of patients	11		16		
Glucose uptake $mg.m^{-2}.min^{-1}$	123	16	163	21	0.166
Total Insulin infused (10 min. prime) $mU.m^{-2}.min^{-1}$	16	0.5	17	0.5	0.052
Total Insulin infused (total, without the prime) $mU.m^{-2}.min^{-1}$	125	11	148	11	0.164

Table 4.21: Euglycemic clamp study results at 3 months. Mean values and SEM are given. P value refers to comparison between the two groups, using t test.

But there was a significant increase in glucose uptake in HMUFA group when the differences between the changes observed at 3 months (compared to base-line) in two diet groups were compared (Table 4.22 and Figure 4.23).

Variable	Control diet 3-0	±SEM	HMUFA diet 3-0	±SEM	Differences: P value
Glucose uptake $mg.m^{-2}.min^{-1}$	-6.7	14	52.6	13.6	**0.007***
Total Insulin infused (10 min. prime) $mU.m^{-2}.min^{-1}$	-0.4	0.2	0.3	0.3	0.097
Total Insulin infused (total, without the prime) $mU.m^{-2}.min^{-1}$	-39	22	-28	15	0.961

Table 4.22: Comparison of differences in euglycemic clamp study between the changes observed in Control diet and HMUFA diet at 3 months (compared to base-line).

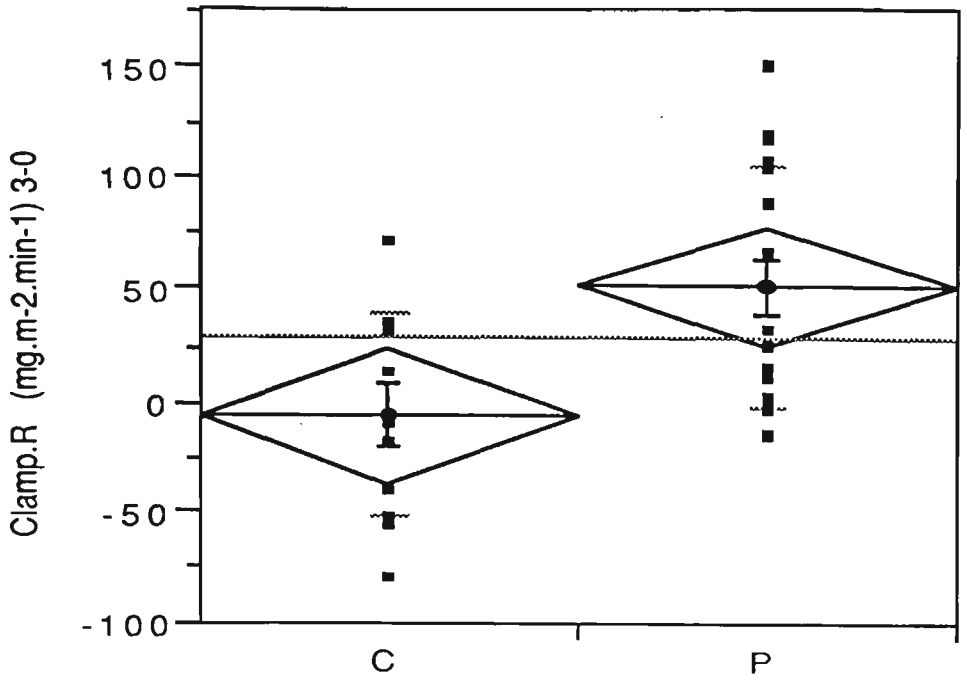

Figure 4.23: Comparison of differences in glucose uptake (clamp.R) between the changes observed in Control diet and HMUFA diet at 3 months (compared to base-line) P=0.007.*

4.6.3 Twelve months time:

At 12 months time there was a significant increase in glucose uptake in HMUFA group, when comparing the two group (Table 4.23 and figure 4.24).

Variable	Control diet 12 months	±SEM	HMUFA diet 12 months	±SEM	Differences: P value
No of patients	11		13		
Glucose uptake $mg.m^{-2}.min^{-1}$	91	14	160	17	**0.009***
Total Insulin infused (10 min. prime) $mU.m^{-2}.min^{-1}$	16	0.5	17	0.4	0.053
Total Insulin infused (total, without the prime) $mU.m^{-2}.min^{-1}$	160	35	193	30	0.204

Table 4.23: Euglycemic clamp study results at 12 months. Mean values and SEM are given. P value refers to comparison between the two groups, using t test.

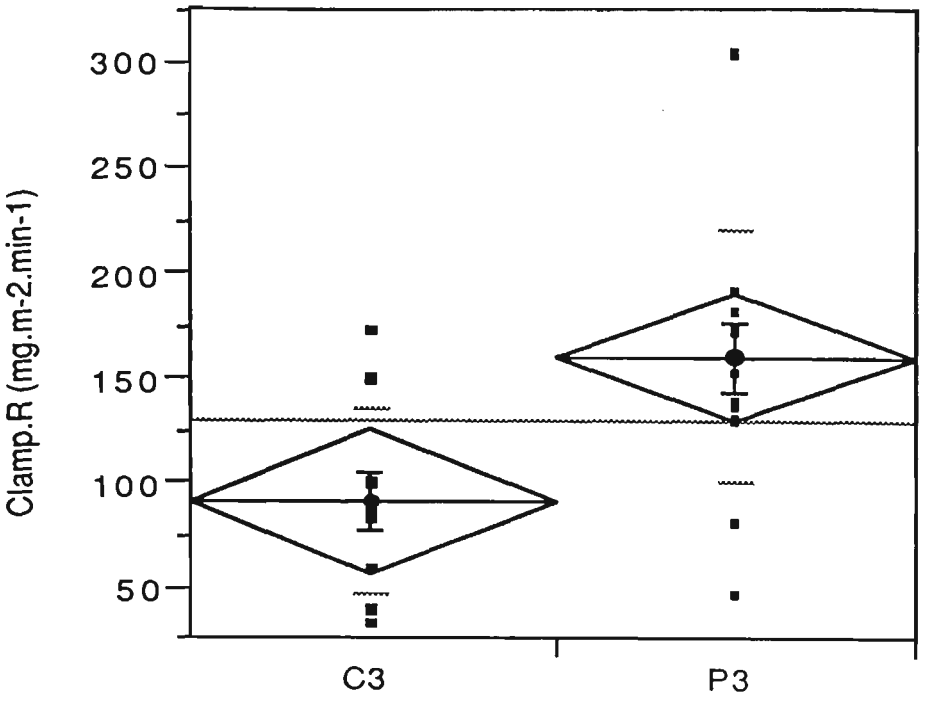

Figure 4.24: Comparison of the differences of the mean ± SEM of glucose uptake (clamp R) between HMUFA diet (P3) and Control diet (C3) at 12 months time (P=0.009).*

In comparing the differences in changes observed at 12 months (compared to base-line), between the two diet group, there was a significant increase of glucose uptake in HMUFA group (P=0.005*), (Table 4.24, Figure 4.24-4.26).

Variable	Control diet 12-0	±SEM	HMUFA diet 12-0	±SEM	Differences: P value
Glucose uptake $mg.m^{-2}.min^{-1}$	-40	22	53	19	**0.005***
Total Insulin infused (10 min. prime) $mU.m^{-2}.min^{-1}$	-6.0	27	20	29	0.828
Total Insulin infused (total, without the prime) $mU.m^{-2}.min^{-1}$	-5.0	27	20	29	0.828

Table 4.24: Comparison of differences in euglycemic clamp study between the changes observed in Control diet and HMUFA diet at 12 months (compared to base-line).

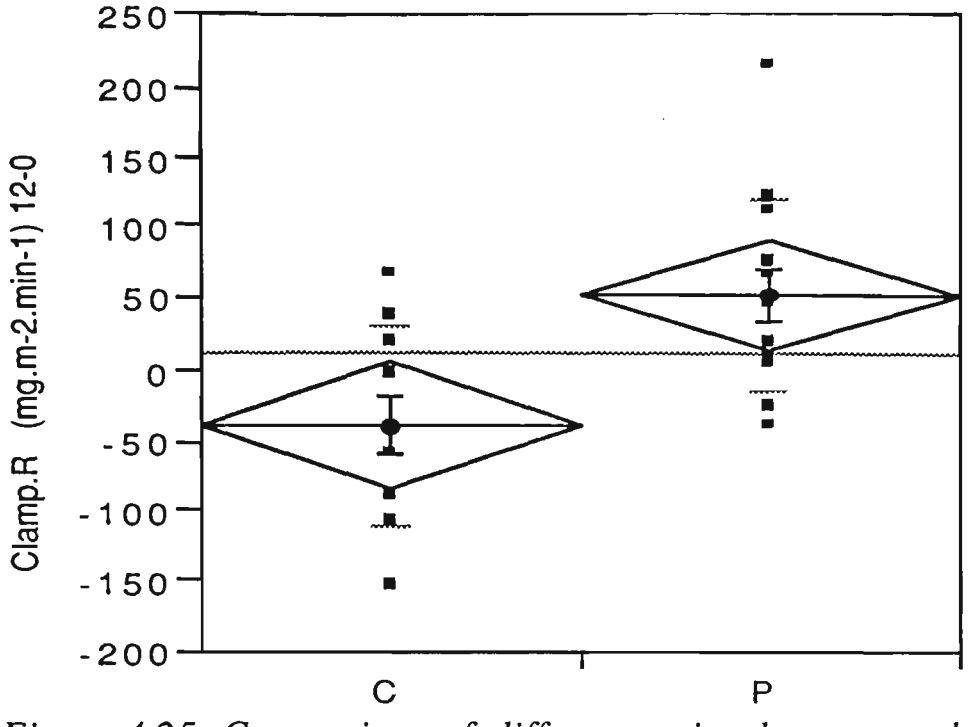

Figure 4.25: Comparison of differences in glucose uptake (clamp R) between the changes observed in Control diet and HMUFA diet at 12 months (compared to base-line) P=0.005.*

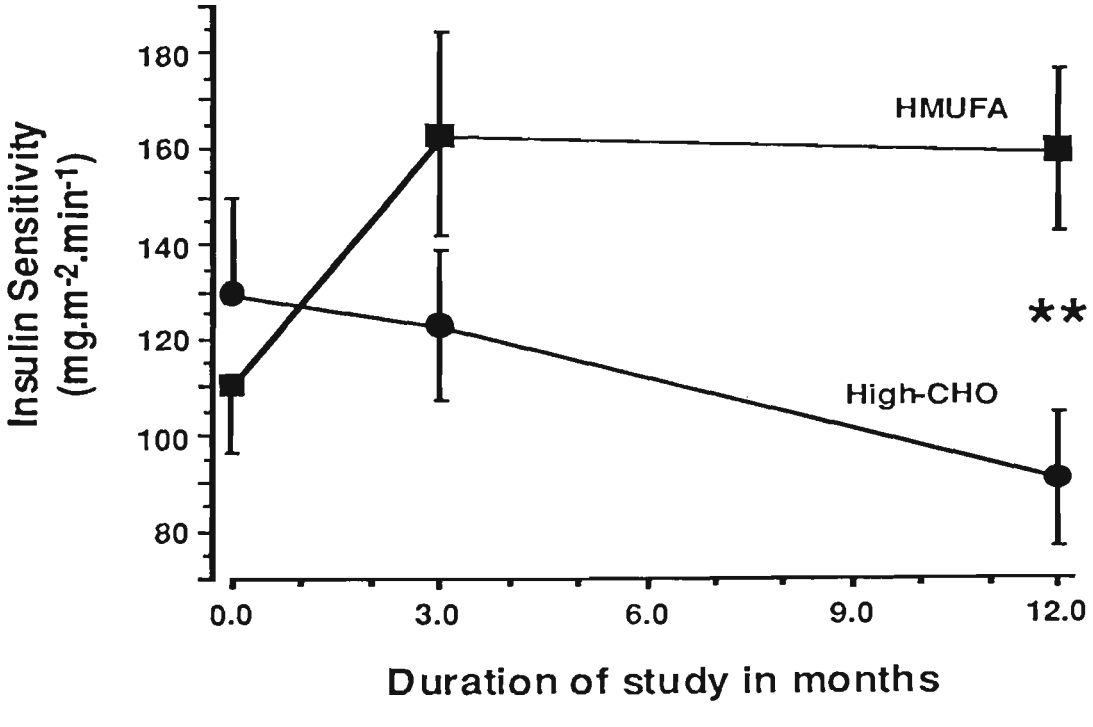

Figure 4.26: Comparison of the mean ± SEM of glucose uptake in Euglycemic clamp study in Control diet (●) and HMUFA (■) diet during the 12 months

In comparison of the differences within the groups, there was a significant increase of glucose uptake in HMUFA group at 3 months and 12 months. No other significant changes observed within the groups (Table 4.25 and Figure 4.27).

	Control (P value)		HMUFA (P value)	
Variable	**3-0**	**12-0**	**3-0**	**12-0**
Glucose uptake $mg.m^{-2}.min^{-1}$	0.791	0.132	**↑0.049***	**↑0.039***
Total Insulin infused (10 min. prime) $mU.m^{-2}.min^{-1}$	0.529	0.512	0.770	0.619
Total Insulin infused (total, without the prime) $mU.m^{-2}.min^{-1}$	0.0943	0.933	0.064	0.559

*Table 4.25: The P value results of comparison within the group at 3 months (3-0) and 12 months (12-0) in euglycemic clamp study. The significant values are shown with *.*

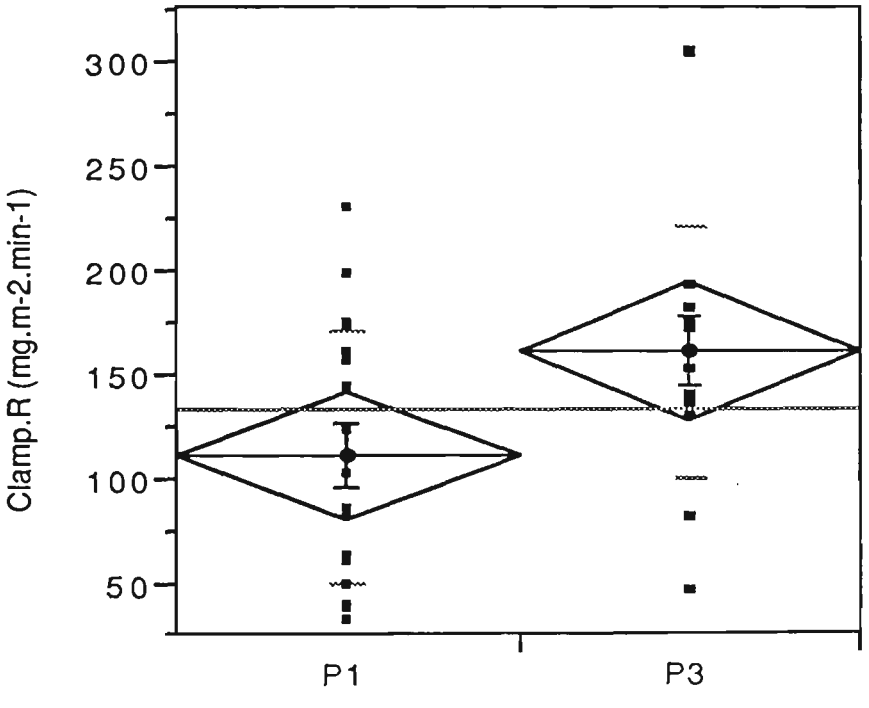

Figure 4.27: Comparison of glucose uptake within HMUFA diet group at 12 months (P=0.039). P1=0 time P3=12 months time*

4.7 Indirect calorimetry:

Indirect calorimetry was measured at 3 months and 12 months but not the 0 time, due to unavailability of the equipment.

4.7.1 Three months:

At 3 months time, there were no significant differences in VCO2 (carbon dioxide oxidation), VO2 (oxygen consumption), RQ (respiratory equation), EE (energy estimate), CHO oxidation, or fat oxidation in fasting (before commencing the clamp study) or during the clamp study, measured by indirect calorimetry, between the Control and HMUFA diet groups (Table 4.26).

Variable	Control diet 3 months	±SEM	HMUFA diet 3 months	±SEM	Differences: P value
VCO2 mL/min (fasting)	213	12	227	10	0.332
VCO2 mL/min (During clamp)	219	10	243	11	0.129
VO2 mL/min (fasting)	252	13	275	12	0.227
VO2, mL/min (During clamp)	244	13	272	11	0.140
RQ mL/min (fasting)	0.84	0.02	0.82	0.01	0.335
RQ mL/min (During clamp)	0.9	0.01	0.9	0.01	0.404
EE mL/min (fasting)	1733	91	1881	84	0.244
EE mL/min (During clamp)	1700	90	1890	80	0.091
CHO oxidation (g/24 hours) (fasting)	164	22	151	21	0.682
CHO oxidation (g/24 hours) (During clamp)	222	15	164	23	0.474
CHO% of total energy (fasting)	40	5.0	33	4.0	0.447
CHO%, of total energy (During clamp)	53	4.0	57	4.0	0.424
fat oxidation (g/24 hours) (fasting)	71	12	91	10	0.198
fat oxidation (g/24 hours) (During clamp)	50	9.0	45	8.0	0.663
fat %, of total energy (fasting)	38	6.0	46	4.0	0.266
fat%, of total energy (During clamp)	26	4.0	22	4.0	0.738

Table 4.26: Indirect calorimetry results at 3 months. Mean values and SEM are given. P value refers to comparison between the two groups, using t test.
VCO2=carbon dioxide oxidation, VO2=oxygen consumption, RQ=respiratory equation, EE=energy estimate.

4.7.2 Twelve months:

At 12 months time (Table 4.27) there were significant differences in fasting VO2 (mL/min) and fasting Energy Expenditure (EE).

Variable	Control diet 12 months	±SEM	HMUFA diet 12 months	±SEM	Differences: P value
VCO2 mL/min (fasting)	211	10	239	9.1	0.060
VCO2 mL/min (During clamp)	219	10	244	10	0.069
VO2 mL/min (fasting)	255	12	289	11	**0.019***
VO2, mL/min (During clamp)	244	11	271	11	0.052
RQ mL/min (fasting)	0.83	0.02	0.83	0.01	0.404
RQ mL/min (During clamp)	0.89	0.01	0.9	0.01	0.399
EE mL/min (fasting)	1743	83	1976	76	**0.025***
EE mL/min (During clamp)	1700	78	1890	79	0.096
CHO oxidation (g/24 hours) (fasting)	149	21	169	12	0.468
CHO oxidation (g/24 hours) (During clamp)	211	25	248	30	0.323
CHO% of total energy (fasting)	36	5.0	32	3.0	0.531
CHO%, of total energy (During clamp)	51	6.0	53	6.0	0.510
fat oxidation (g/24 hours) (fasting)	80	12	91	7.0	0.468
fat oxidation (g/24 hours) (During clamp)	38	7.0	40	7.0	0.858
fat %, of total energy (fasting)	42	6.0	44	2.0	0.668
fat%, of total energy (During clamp)	20	3.0	19	3.6	0.909

Table 4.27: Indirect calorimetry results at 12 months. Mean values and SEM are given. P value refers to comparison between the two groups, using t test.
VCO2=carbon dioxide oxidation, VO2=oxygen consumption, RQ=respiratory equation, EE=energy estimate.

There were not any significant changes within the group at 12 months in HMUFA diet or Control diet (Table 4.28).

	Control (P value)	HMUFA (P value)
Variable	12-3	12-3
VCO2 mL/min (fasting)	0.901	0.388
VCO2 mL/min (During clamp)	0.941	0.955
VO2 mL/min (fasting)	0.888	0.402
VO2, mL/min (During clamp)	0.973	0.994
RQ mL/min (fasting)	0.331	0.651
RQ mL/min (During clamp)	0.330	0.0560
EE mL/min (fasting)	0.936	0.423
EE mL/min (During clamp)	0.967	0.994
CHO oxidation (g/24 hours) (fasting)	0.624	0.490
CHO oxidation (g/24 hours) (During clamp)	0.735	0.363
CHO% of total energy (fasting)	0.609	0.883
CHO%, of total energy (During clamp)	0.825	0.562
fat oxidation (g/24 hours) (fasting)	0.584	0.986
fat oxidation (g/24 hours) (During clamp)	0.256	0.635
fat %, of total energy (fasting)	0.570	0.685
fat%, of total energy (During clamp)	0.254	0.585

Table 4.28: The P value results of comparison within the group at 12 months (compare to 3 months) in Euglycemic clamp study.
VCO2=carbon dioxide oxidation, VO2=oxygen consumption, RQ=respiratory equation, EE=energy estimate.

4.8 Diet:

4.8.1 Daily energy intake:

4.8.1.1 Base-line:

At Base-line (0 time), there were no significant differences in daily energy intake between the Control group and HMUFA group (Table 4.29).

Variable	Control diet 0 time	±SEM	HMUFA diet 0time	±SEM	Differences P value
Basic metabolic rate Kcal	1775	61	1819	61	0.610
Estimate intake	1930	145	1845	105	0.636
Fat, E%	32%	2.0	33.2	1.3	0.694
g	73	8	69.5	5.9	0.720
CHO E%	45.9%	2.2	44	1.3	0.896
g	219	16	207	12	0.547
Simple CHO%	0.31	0.03	0.34	0.02	0.145
Complex CHO%	0.69	0.03	0.66	0.02	0.145
Dietary fibre, g	26.7	2.5	24.7	1.6	0.499
Total sugars, g	69	9	68.6	5.0	0.632
Starch, g	145	14	137.5	9.9	0.657
Sat. fat, % total fat	39.8%	0.9	38.8	0.9	0.483
g	30	5.0	24.7	2.1	0.573
Poly. fat, % total fat	21.7%	1.5	20.1	0.9	0.368
g	13.8	1.8	12.1	1.1	0.436
Mono. fat, % total fat	38.7%	1.3	41.2	1.0	0.179
g	27	3.1	26.7	2.8	0.986
Mono:sat:ratio	0.99:1	0.04	1.08:1	0.04	0.183
Poly:sat. ratio	0.57:1	0.05	0.53:1	0.03	0.535
Canola% total fat	3.1	1.6	9.2	2.9	0.071
g	2.8	1.5	6.9	2.8	0.189
Canola n-3, g	0.3	0.2	0.7	0.3	0.189
Total n-6, g	13.4	1.8	11.3	1.1	0.319
n-6:n-3 ratio	44.6		16.1		
Cholesterol, mg	272	41	227	15	0.682
Protein E%	21%	1.0	21.2%	0.8	0.854
g	96	7.3	91	4.3	0.559
Alcohol E%	0.67%	0.3	1.6%	1.0	0.837
g	2.4	1.2	4.6	3.5	0.763

Table 4.29: Daily energy intake in the diet from four days food record at baseline (0 time). Mean values and SEM are given. P value refers to comparison between the two groups, using t test.

4.8.1.2 Three months:

At 3 months time, there were significant differences in saturated fat intake (%), monounsaturated fat intake (%), mono:sat ratio, poly:sat ratio, canola intake (% and g), and n-3 intake from canola the Control group and HMUFA group (Table 4.34 and Figures 4.28 and 4.29). These changes were noted as the HMUFA group increased their intake of monounsaturated fat and decreased their saturated fat intake.

Variable	Control diet 3 months	±SEM	HMUFA diet 3 months	±SEM	Differences: P value
Basic metabolic rate Kcal	1760	64	1790	61	0.735
Estimate intake	1839	138	1601	103	0.175
Fat, E%	28.9%	2.2	31.1%	1.2	0.384
g	62	6	55.2	4.7	0.420
CHO E%	46.1%	3.1	46%	1.2	0.742
g	208	20	188	13	0.716
Simple CHO%	0.33	0.04	0.36	0.02	0.098
Complex CHO%	0.67	0.04	0.64	0.02	0.098
Dietary fibre, g	25.7	2.7	23.9	1.9	0.991
Total sugars, g	69	9	70.9	7.7	0.751
Starch, g	138	16	115.4	6.9	0.360
Sat. fat, % total	40%	1.1	32%	1.3	**0.000***
fat g	23	3	15.8	1.5	0.360
Poly. fat, % total	20.2%	1.5	20.7%	0.9	0.775
fat g	10	1.0	10.2	0.9	0.880
Mono. fat, % total fat	39.8%	1.1	47.2%	1.0	**0.000***
g	23	2.5	23.6	2.2	0.750
Mono:sat:ratio	1.01:1	0.04	1.54:1	0.09	**0.000***
Poly:sat. ratio	0.53:1	0.05	0.68:1	0.05	**0.015***
Canola% total fat	5.5	2.5	27	3.4	**0.000***
g	3.6	1.8	15.3	2.7	**0.001***
Canola n-3, g	0.4	0.2	1.5	0.3	**0.001***
Total n-6, g	9.7	1.0	9.2	0.9	0.737
n-6:n-3 ratio	24.3		6.1		
Cholesterol, mg	291	44	203	16	0.065
Protein E%	22.0%	1.1	22.0%	0.8	0.950
g	96.4	7.6	83.8	4.9	0.171
Alcohol E%	2.9%	1.3	1.1%	0.6	0.074
g	9.0	4.4	2.9	1.6	0.068

Table 4.30: Daily energy intake in the diet from four days food record at 3 months. Mean values and SEM are given. P value refers to comparison between the two groups, using t test.

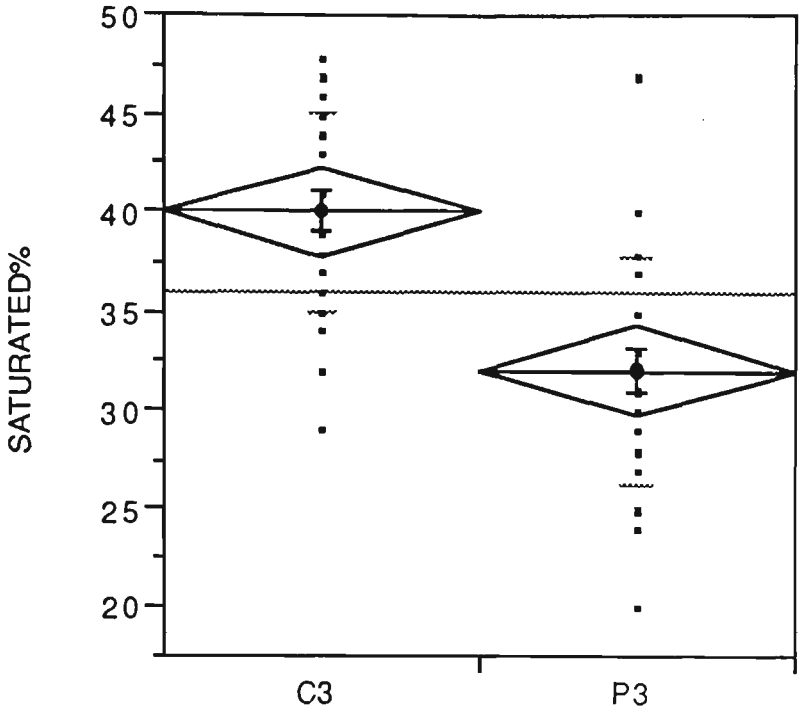

Figure 4.28: Comparison of the distribution mean ± SEM of saturated intake% (energy from fat) at 3 months time between Control (C3) and HMUFA (P3) diet (P=0.000).*

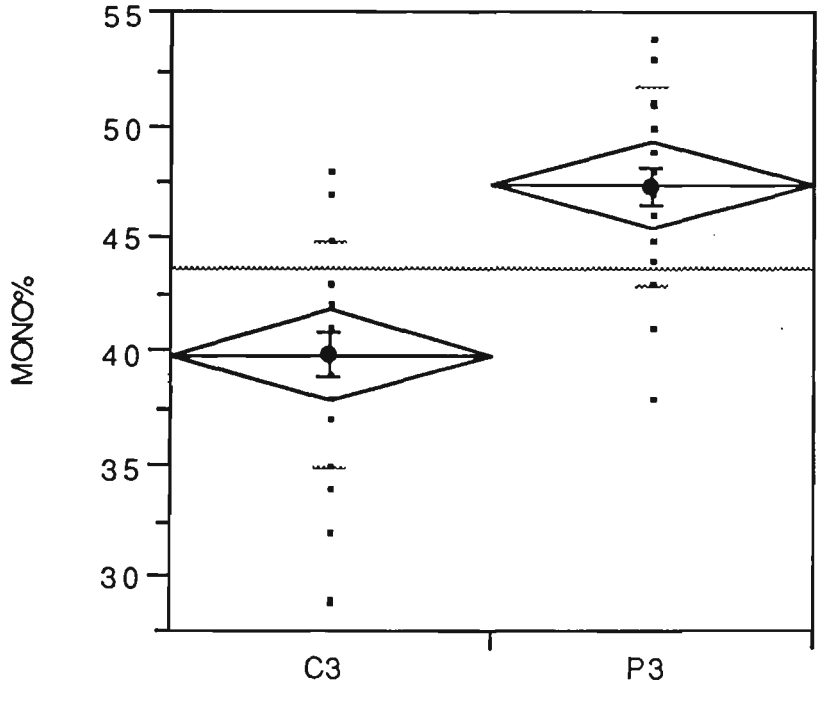

Figure 4.29: Comparison of the means of monounsaturated intake% (energy from fat) at 3 months time between Control (C3) and HMUFA (P3) diet (P=0.000).*

There were significant changes in saturated fat intake (% and g), monounsaturated fat intake (%), monounsaturated fat:saturated fat ratio, polyunsaturated:saturated fat ratio, canola intake (%), and protein intake (g) when the differences between the changes observed at 3 months (compared to base-line) in two diet groups were compared (Table 4.31).

Variable	Control diet 3-0	±SEM	HMUFA diet 3-0	±SEM	Differences P value
Basic metabolic rate Kcal	-17	6.0	-26	8.0	0.959
Estimate intake	-54.8	125	-258	90	0.053
Fat, E%	-3.2	1.3	-2.5	1.4	0.691
g	-9.7	6.0	-15.4	5.2	0.255
CHO E%	0.00	1.9	1.1	1.7	0.953
g	-8.4	16.4	-24	11	0.424
Simple CHO%	0.01	0.02	0.02	0.02	0.755
Complex CHO%	0.01	0.02	-0.02	0.02	0.755
Dietary fibre, g	-1.5	1.8	-0.8	1.5	0.916
Total sugars, g	-1.0	7.3	1.2	5.5	0.812
Starch, g	-3.3	12.3	-25.8	8.3	0.138
Sat. fat, % total fat	0.5	1.6	-7.3	1.5	**0.001***
g	-6.2	4.5	-9.4	1.8	0.016
Poly. fat, % total fat	-1.7	1.2	0.9	1.1	0.128
g	-3.5	1.7	-2.1	0.9	0.935
Mono. fat, % total fat	1.1	1.1	6.4	1.4	**0.006***
g	-3.5	2.5	-3.3	2.7	0.707
Mono:sat:ratio	0.02	0.06	0.48	0.09	**0.000***
Poly:sat. ratio	-0.04	0.06	0.17	0.06	**0.018***
Canola% total fat	2.0	1.6	16.4	4.0	**0.001***
g	0.7	0.9	7.0	4.2	0.099
Canola n-3, g	0.1	0.1	0.7	0.4	0.099
Total n-6, g	-3.7	1.7	-2.5	1.7	0.628
n-6:n-3 ratio	-20.3		-10		
Cholesterol, mg	22.1	54	-30.5	22	0.149
Protein E%	1.0	1.1	0.7	0.9	0.813
g	2.4	6.2	-8.9	4.2	**0.034***
Alcohol E%	2.4	1.3	0.5	0.3	0.164
g	7.2	4.5	1.6	1.1	0.137

Table 4.31: Comparison of differences in daily energy intake in the diet from four days food record between the changes observed in Control diet and HMUFA diet at 3 months (compared to base-line).

4.8.1.3 Six months:

At 6 months time, there were significant differences in sat. fat intake (%), MUFA intake (%),mono:sat fat:ratio and canola intake, n-3 and from canola between the Control group and HMUFA group (Table 4.32 and Figures 4.30 and 4.31). These changes were noted as the HMUFA group increased their intake of MUFA and decreased their saturated fat intake.

Variable	Control diet 6 months	±SEM	HMUFA diet 6 months	±SEM	Differences: P value
Basic metabolic rate,	1756	68	1794	57	0.667
Estimate intake	1866	146	1767	96	0.571
Fat, E%	30%	2.0	31.3%	1.8	0.502
g	62	6	72.8	9.7	0.784
CHO, E%	47%	2.6	47%	1.8	0.648
g	218	21	195	14	0.865
Simple CHO%	0.33	0.04	0.41	0.06	0.085
Complex CHO%	0.67	0.04	0.59	0.06	0.085
Dietary fibre, g	26.4	2.6	27.1	1.5	0.812
Total sugars, g	71	10	80.7	7.5	0.127
Starch, g	145	16	122.6	11	0.397
Sat. fat, % total fat	36.6%	1.2	30.7%	1.8	**0.009***
g	21	2.0	18	1.7	0.375
Poly. fat, % total fat	21.9%	1.5	21%	1.0	0.643
g	12	1.3	13.2	1.8	0.572
Mono. fat, % total fat	41.7%	1.4	48.3%	1.2	**0.001***
g	24	2.4	26.5	3.3	0.480
Mono:sat:ratio	1.17:1	0.06	1.71:1	0.09	**0.000***
Poly:sat. ratio	0.63:1	0.06	0.76:1	0.05	0.127
Canola% total fat	8.6	3.2	32	3.7	**0.000***
g	5.1	2.6	23.3	3.7	**0.001***
Canola n-3, g	0.5	0.3	2.2	0.4	**0.001***
Total n-6, g	11.4	1.3	10.9	1.9	0.819
n-6:n-3 ratio	22.8		4.95		
Cholesterol, mg	208	28	186	15	0.486
Protein, E%	21%	0.9	20.8%	0.5	0.892
g	93.1	7.2	87.7	4.7	
Alcohol, E%	2.7%	1.3	0.7%	0.4	0.123
g	8.6	4.2	2.0	1.3	0.124

Table 4.32: Daily energy intake in the diet from four days food record at 6 months. Mean values and SEM are given. P value refers to comparison between the two groups, using t test.

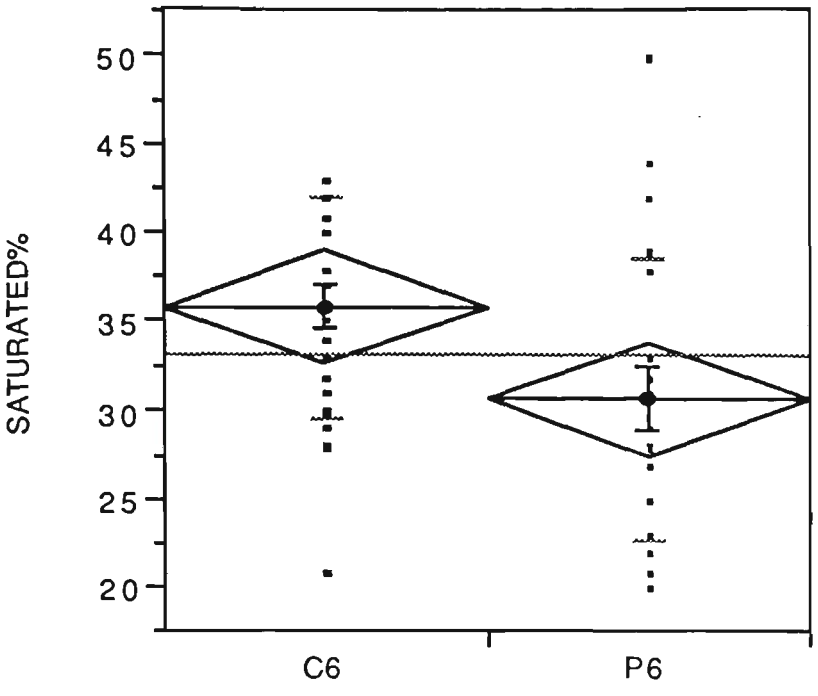

Figure 4.30: Comparison of the mean ± SEM of saturated intake% (energy from fat) at 6 months time between Control (C6) and HMUFA (P6) diet (P=0.009).*

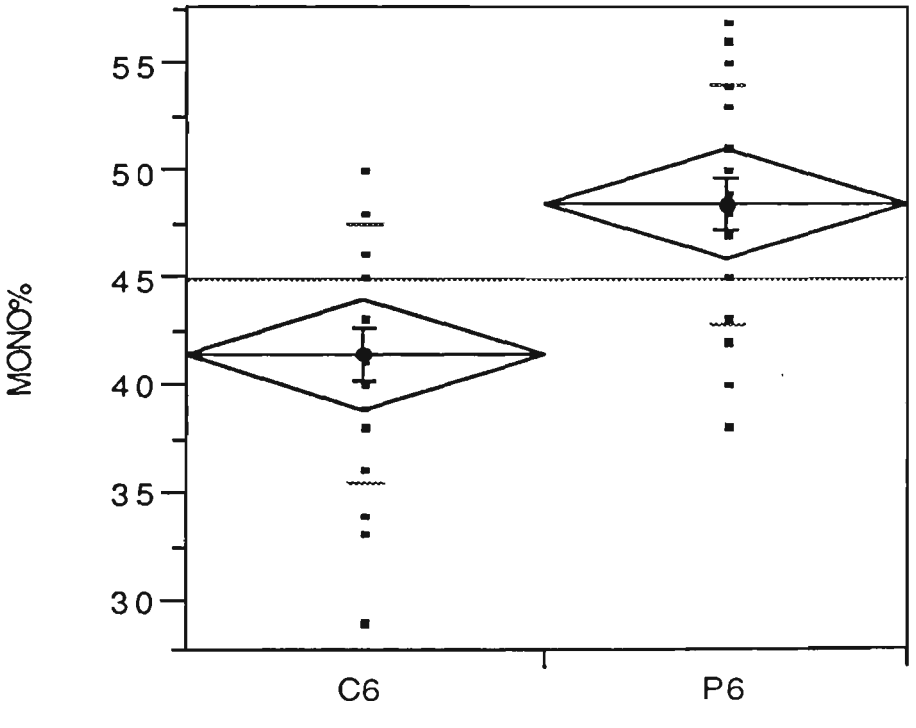

Figure 4.31: Comparison of the distribution mean ± SEM of monounsaturated intake% (energy from fat) at 6 months time between Control (C6) and HMUFA (P6) diet (P=0.001).*

There were significant changes in monounsaturated fat:saturated fat:ratio, canola intake (% and g), and n-3 intake from canola when the differences between the changes observed at 6 months (compared to base-line) in two diet groups were compared (Table 4.33). No other significant changes were noted.

Variable	Control diet 6-0	±SEM	HMUFA diet 6-0	±SEM	Differences: P value
Basic metabolic rate,	-51.4	39	16	95	0.574
Estimate intake	-55	143	26	197	0.743
Fat, E%	-2.6	2.5	-3.2	1.9	0.841
g	-9.6	8.0	3.7	10	0.474
CHO, E%	1.2	2.9	3.3	2.3	0.865
g	-3.0	18	1.6	27	0.743
Simple CHO%	0.01	0.05	0.23	0.2	0.653
Complex CHO%	0.01	0.05	0.23	0.2	0.653
Dietary fibre, g	-1.1	2.5	3.9	2.8	0.339
Total sugars, g	0.4	12.7	11.4	1.0	0.913
Starch, g	-0.3	13	-1.9	21	0.601
Sat. fat, % total fat	-4.0	1.8	-7.7	1.9	0.167
g	-8.9	5.0	-2.3	5.3	0.780-
Poly. fat, % total fat	1.0	2.6	1.6	1.3	0.674
g	-1.8		1.4	2.3	0.633
Mono. fat, % total fat	3.4	1.6	6.5	1.2	0.055
g	-2.4	2.8	-0.3	4.5	0.743
Mono:sat:ratio	0.2	0.1	0.6	0.1	**0.005***
Poly:sat. ratio	0.13	0.1	0.23	0.1	0.238
Canola% total fat	7.5	2.9	21	4.8	**0.016***
g	3.8	2.1	15.6	3.8	**0.007***
Canola n-3, g	0.4	0.2	1.6	0.4	**0.007***
Total n-6, g	-2.1	2.3	0.8	2.2	0.378
n-6:n-3 ratio	-21.8		-11.15		
Cholesterol mg	-62	38	-32	23	0.642
Protein, E%	-0.2	1.5	-0.5	0.9	0.850
g	-1.8	6.9	1.1	8.4	0.795
Alcohol, E%	1.9	1.2	0.22	1.7	0.645
g	6.3	4.0	1.0	5.5	0.956

Table 4.33: Comparison of differences in daily energy intake in the diet from four days food record between the changes observed in Control diet and HMUFA diet at 6 months (compared to base-line).

4.8.1.4 Twelve months:

At 12 months time, there were significant differences in saturated fat intake (%), monounsaturated fat intake (%), monounsaturated fat:saturated fat ratio, and polyunsaturated:saturated fat ratio, canola intake (% and g), and n-3 intake from canola, between the Control group and HMUFA group (Table 4.34 and Figures 4.32 to 4.45).

Variable	Control diet 12 months	±SEM	HMUFA diet 12 months	±SEM	Differences: P value
Basic metabolic rate, Kcal	1744	66	1780	57	0.681
Estimate intake Kcal	1958	130	1753	101	0.226
Fat, E%	31	1.7	33.9%	1.8	0.374
g	64	6.0	67.9	6.5	0.708
CHO, E%	43.5%	3.1	43%	1.4	0.715
g	218	19	188	9.0	0.188
Simple CHO%	0.33%	0.03	0.33%	0.02	0.667
Complex CHO%	0.67%	0.03	0.67%	0.02	0.667
Dietary fibre, g	27.3	2.7	24.9	2.0	0.468
Total sugars, g	72	8	62.2	5.0	0.273
Starch, g	144	16	121.2	6.8	0.189
Sat. fat, % total fat	38%	0.8	29.4%	1.1	**0.000***
g	24	2.0	17.8	1.7	0.375
Poly. fat, % total fat	19.7%	1.3	21%	0.7	0.391
g	12	1.3	13.1	1.6	0.715
Mono. fat, % total fat	42.3%	1.0	49.6%	1.0	**0.000***
g	25	2.7	30.4	3.1	0.273
Mono:sat:ratio	1.13:1	0.04	1.75:1	0.1	**0.000***
Poly:sat. ratio	0.53:1	0.04	0.74:1	0.04	**0.001***
Canola% total fat	6.1	2.4	31	4.6	**0.000***
g	4.8	2.1	20.1	3.3	**0.000***
Canola n-3, g	0.5	0.2	2.0	0.3	**0.000***
Total n-6, g	11.5	1.3	10.9	1.7	0.789
n-6:n-3 ratio	23		5.45		
Cholesterol, mg	254	31	222	20	0.557
Protein, E%	22.3%	1.9	21.7	0.7	0.447
g	102.8	9.2	90.8	4.9	0.397
Alcohol, E%	2.7%	1.3	1.6%	0.8	0.439
g	9.0	4.6	4.0	1.9	0.403

Table 4.34: Daily energy intake in the diet from four days food record at 12 months. Mean values and SEM are given. P value refers to comparison between the two groups, using t test.

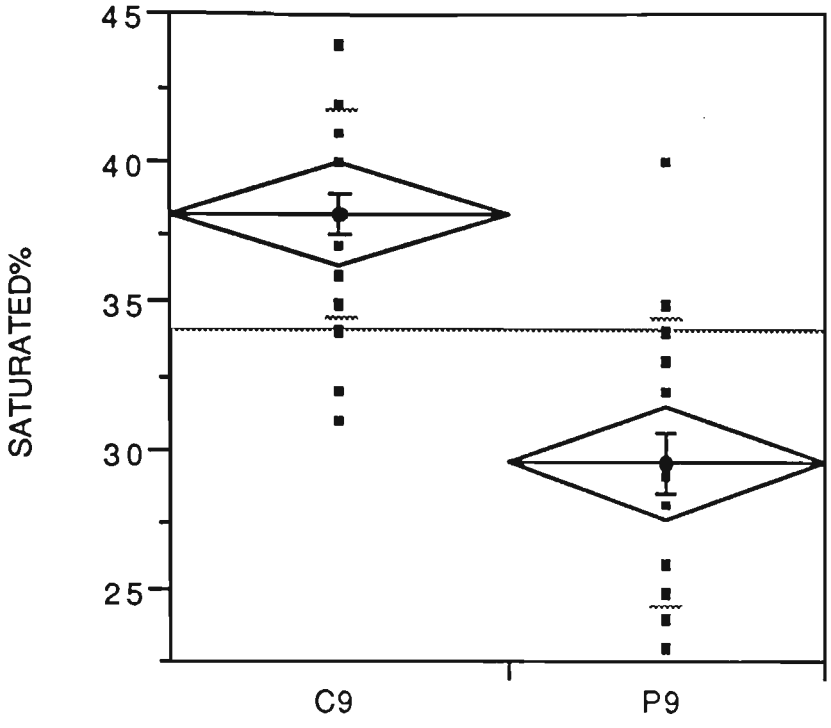

Figure 4.32: Comparison of the mean ± SEM of saturated intake% (energy from fat) at 12 months time between Control (C9) and HMUFA (P9) diet (P=0.000).*

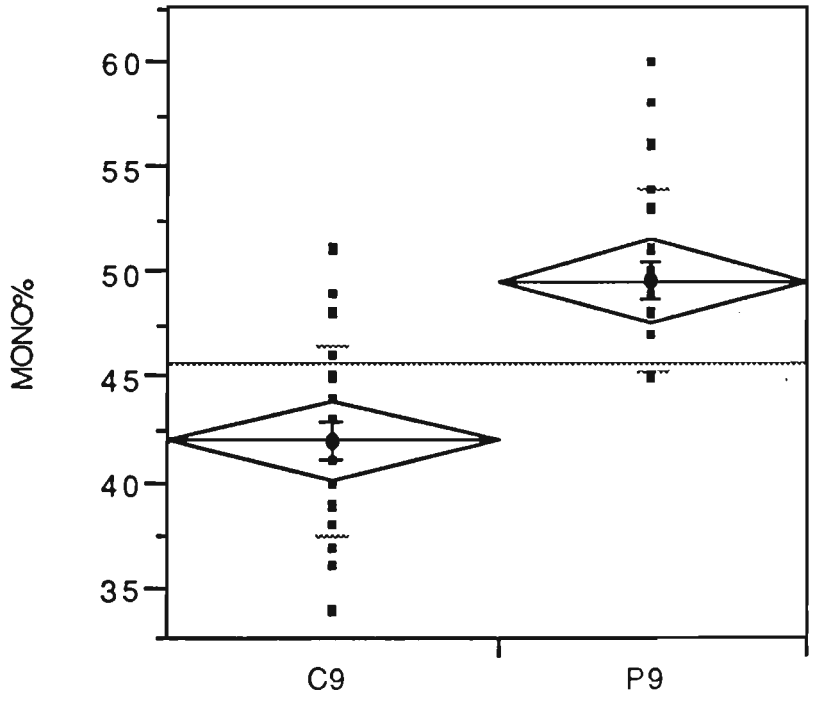

Figure 4.33: Comparison of the mean ± SEM of monounsaturated intake% (energy from fat) at 12 months time between Control (C9) and HMUFA (P9) diet (P=0.000).*

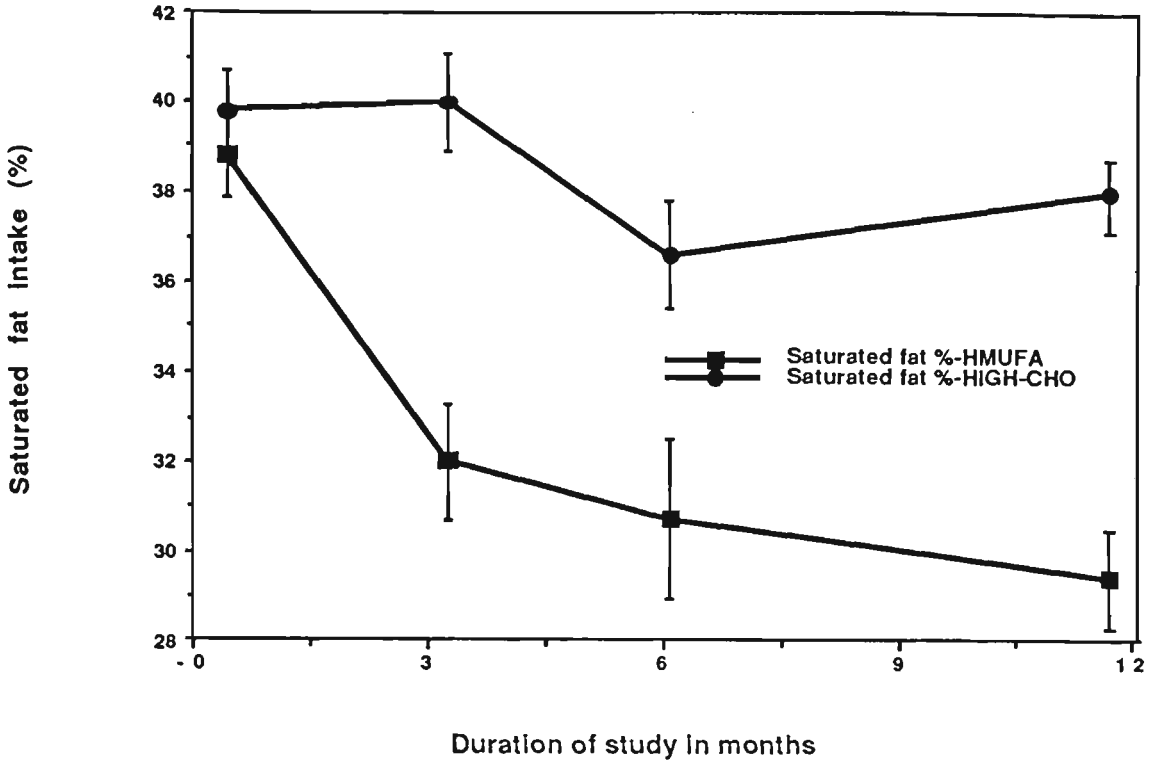

Figure 4.34: Comparison of the mean ± SEM of saturated fat intakes (%)(energy from fat) during 12 months. Control diet (●) and HMUFA (■) diet

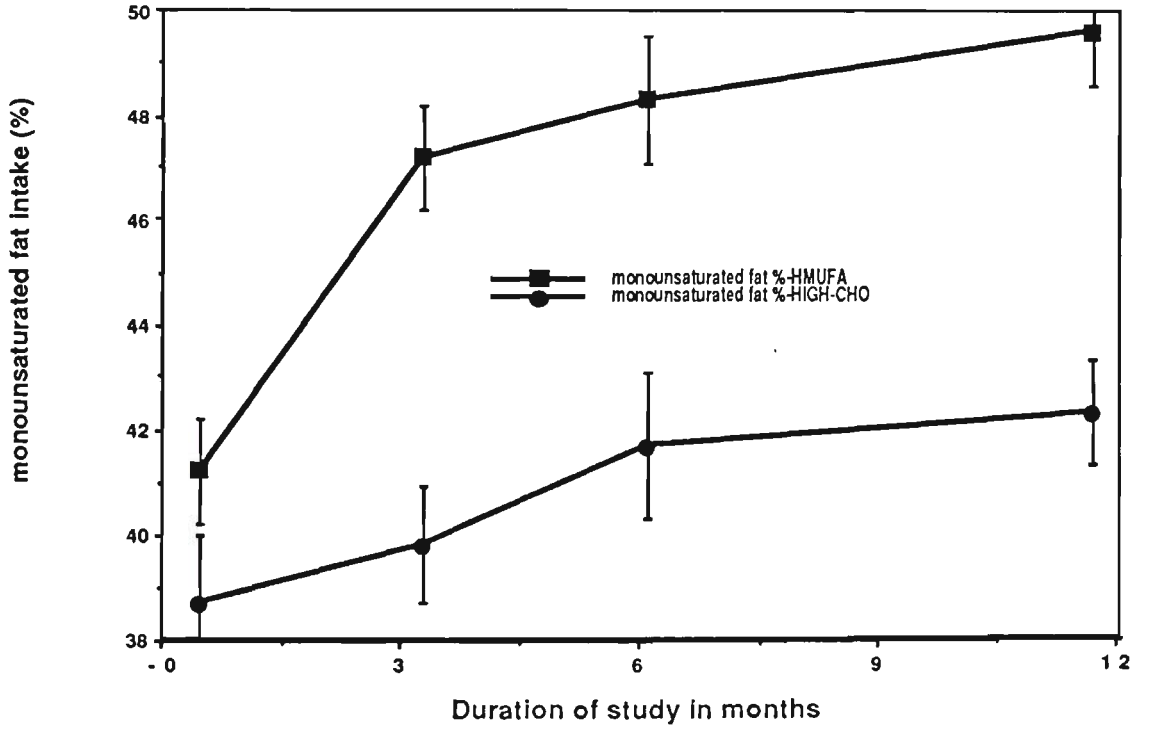

Figure 4.35: Comparison of the mean ± SEM of mono.unsaturated fat intakes (%)(energy from fat) during 12 months. Control diet (●) and HMUFA (■) diet.

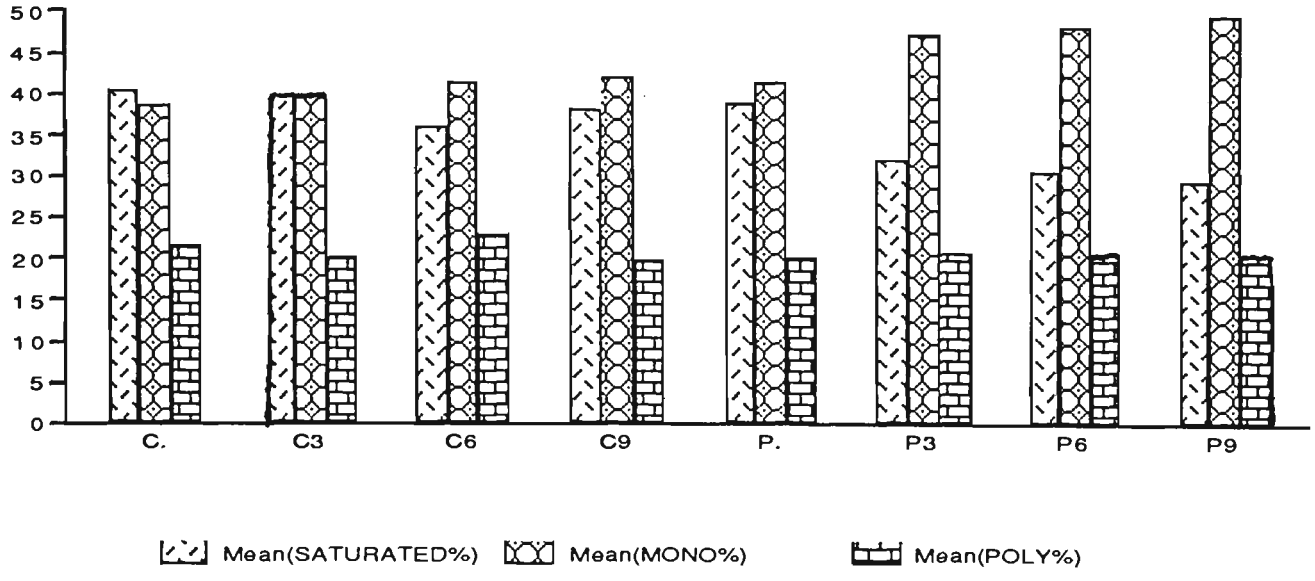

Figure 4.36: Comparison of the mean ± SEM of Sat.F%, Mono.unsat.F%, and Poly.unsat.F% (energy from fat) during 12 months in Control diet (C.-C3-C6-C9) and HMUFA diet (P.-P3-P6-P9).
C.&P.= 0 time, C3&P3= 3 months, C6&P6= 6 months, C9&P9= 12 months.

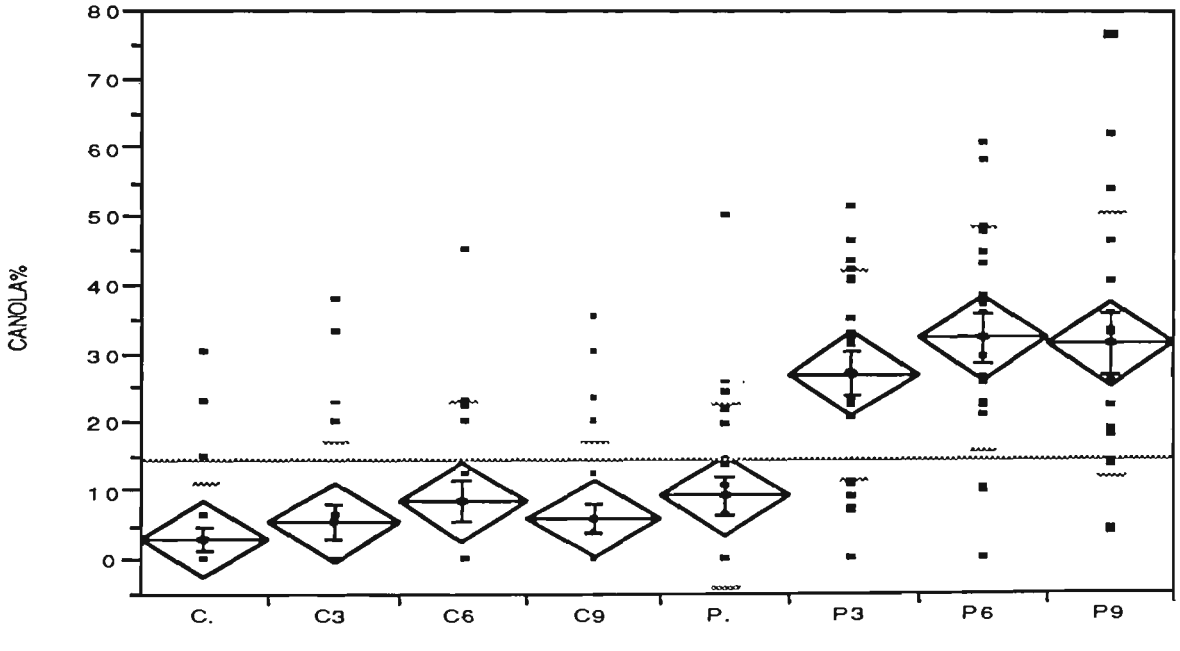

Figure 4.37: Comparison of the mean of canola% (energy from fat) intake during the study in HMUFA group (P.=0 time, P3= 3 months, P6= 6 months, P9= 12 months) and Control group (C.=0 time, C3= 3 months, C6= 6 months, C9= 12 months..

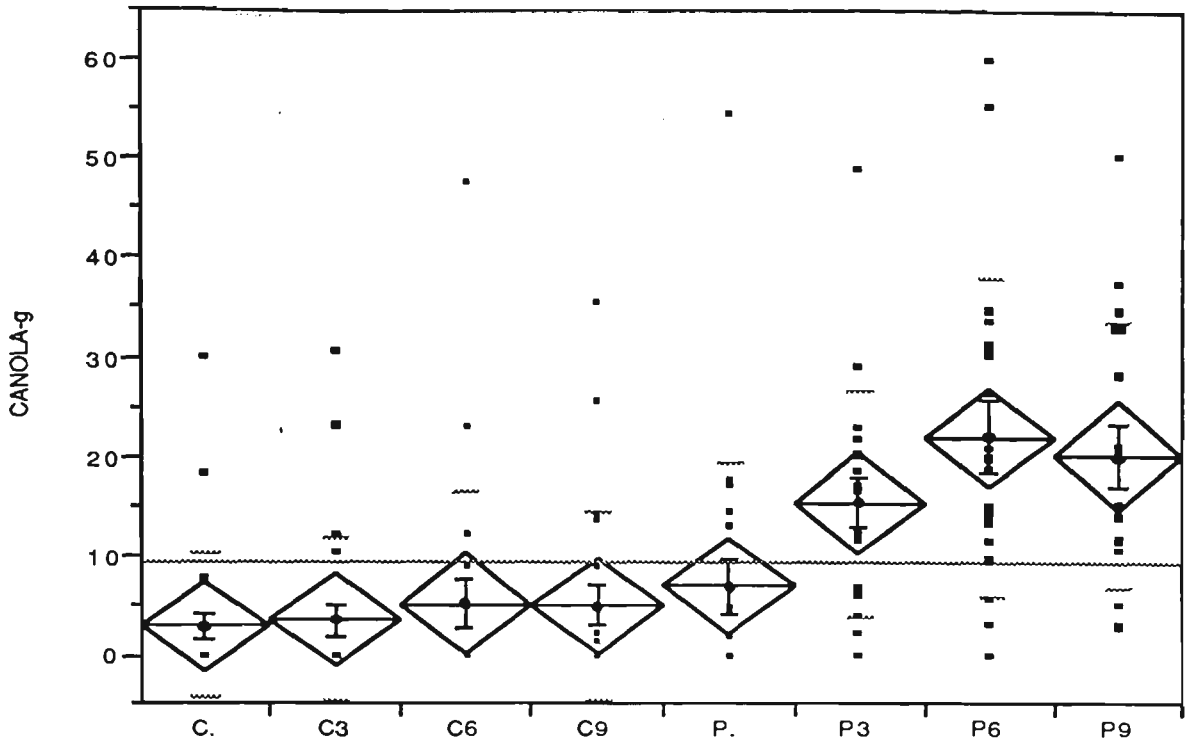

Figure 4.38: Comparison of the mean of canola g intake during the study in HMUFA group (P.=0 time, P3= 3 months, P6= 6 months, P9= 12 months) and Control group (C.=0 time, C3= 3 months, C6= 6 months, C9= 12 months..

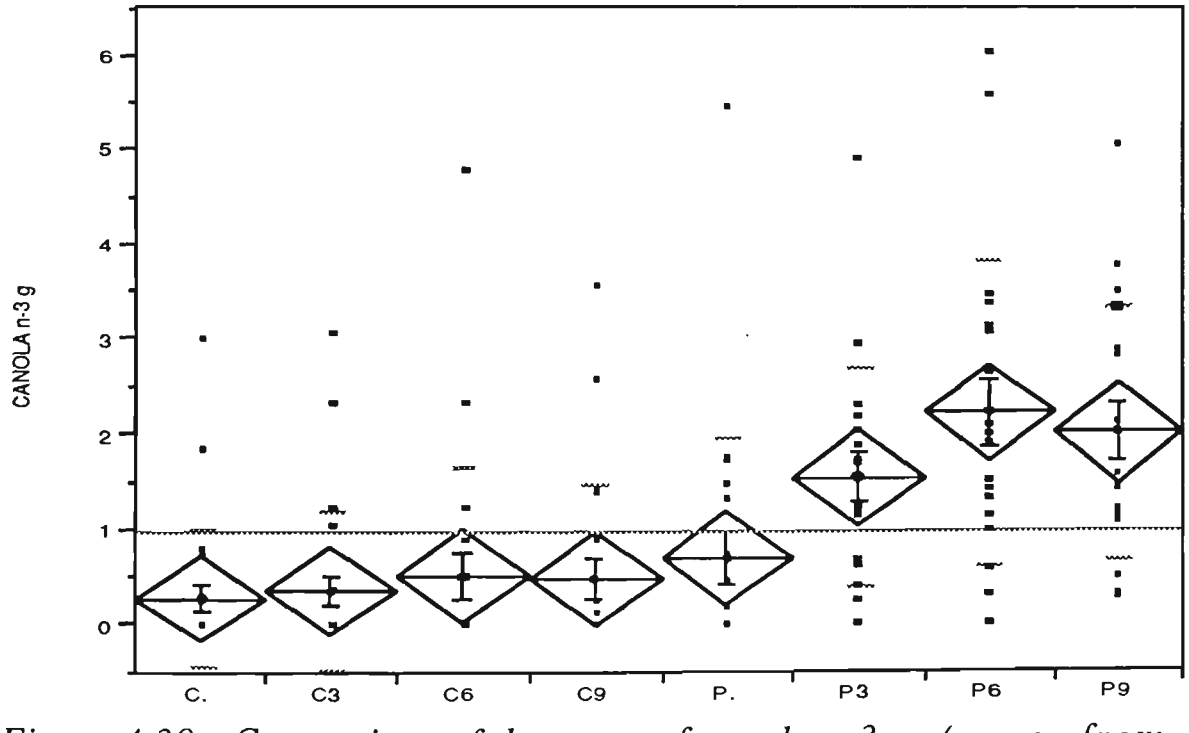

Figure 4.39: Comparison of the mean of canola n-3 g (energy from fat) intake during the study in HMUFA group (P.=0 time, P3= 3 months, P6= 6 months, P9= 12 months) and Control group (C.=0 time, C3= 3 months, C6= 6 months, C9= 12 months

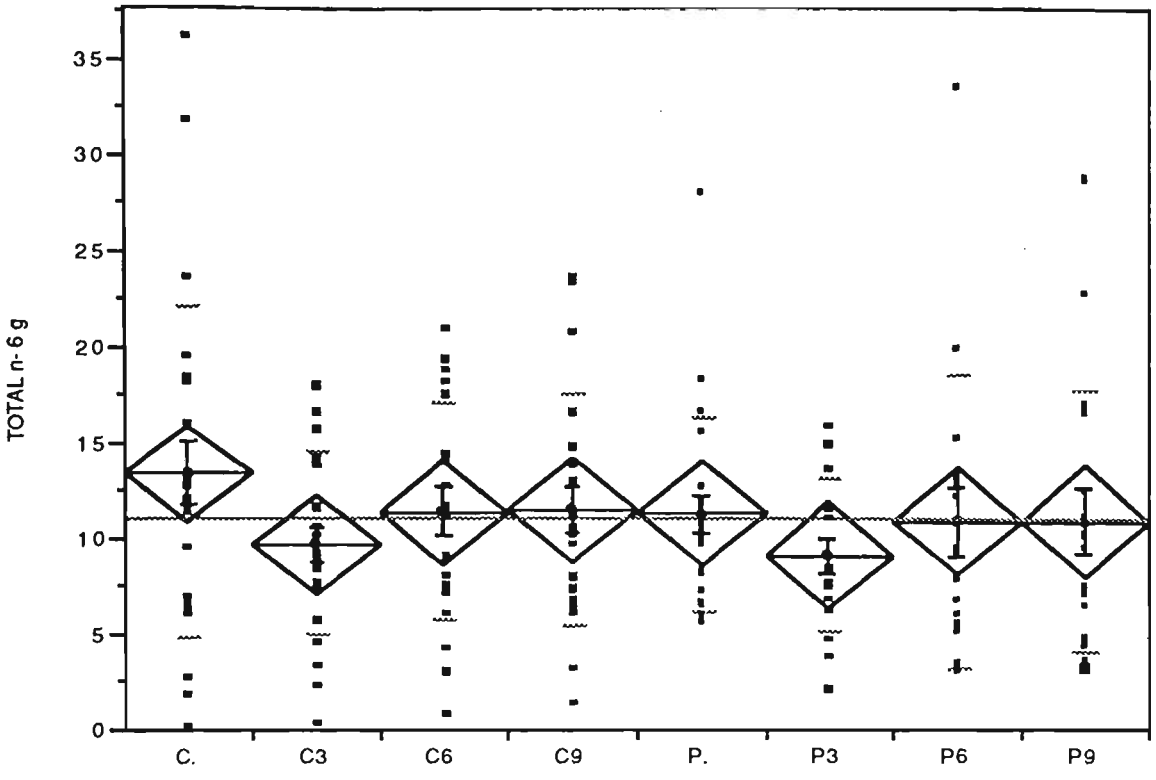

Figure 4.40: Comparison of the mean of total n-6 g (energy from fat) intake during the study in HMUFA group (P.=0 time, P3= 3 months, P6= 6 months, P9= 12 months) and Control group (C.=0 time, C3= 3 months, C6= 6 months, C9= 12 months

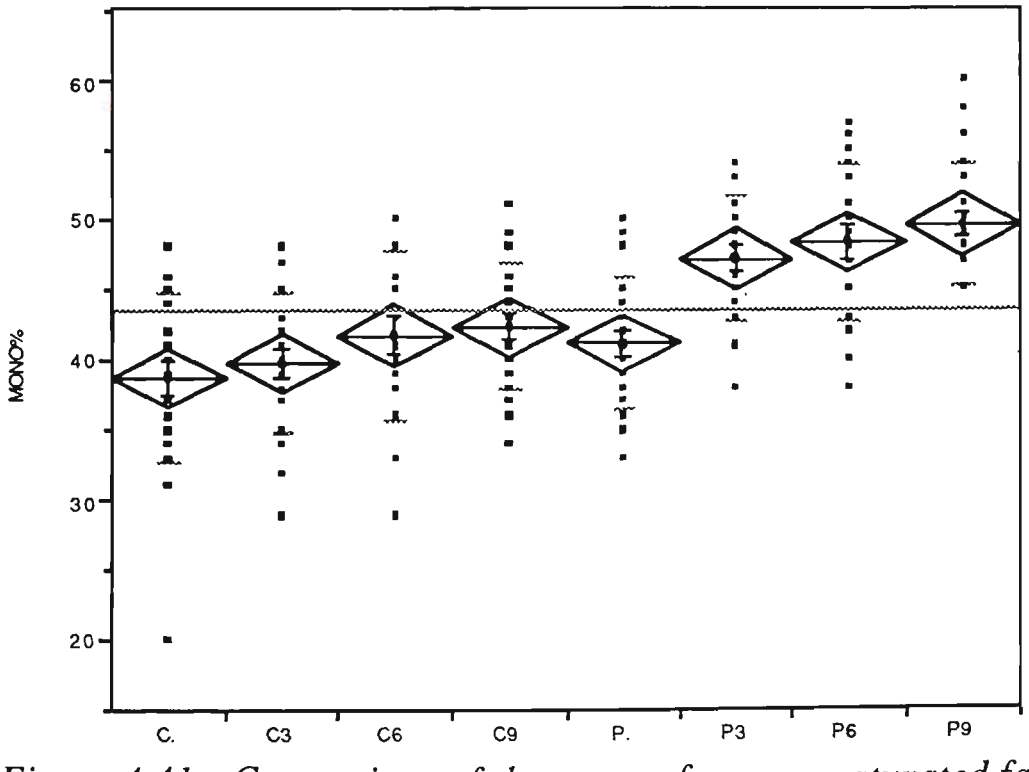

Figure 4.41: Comparison of the mean of monounsaturated fat intake (%)(energy from fat) during the study in HMUFA group (P.=0 time, P3= 3 months, P6= 6 months, P9= 12 months) and Control group (C.=0 time, C3= 3 months, C6= 6 months, C9= 12 months.

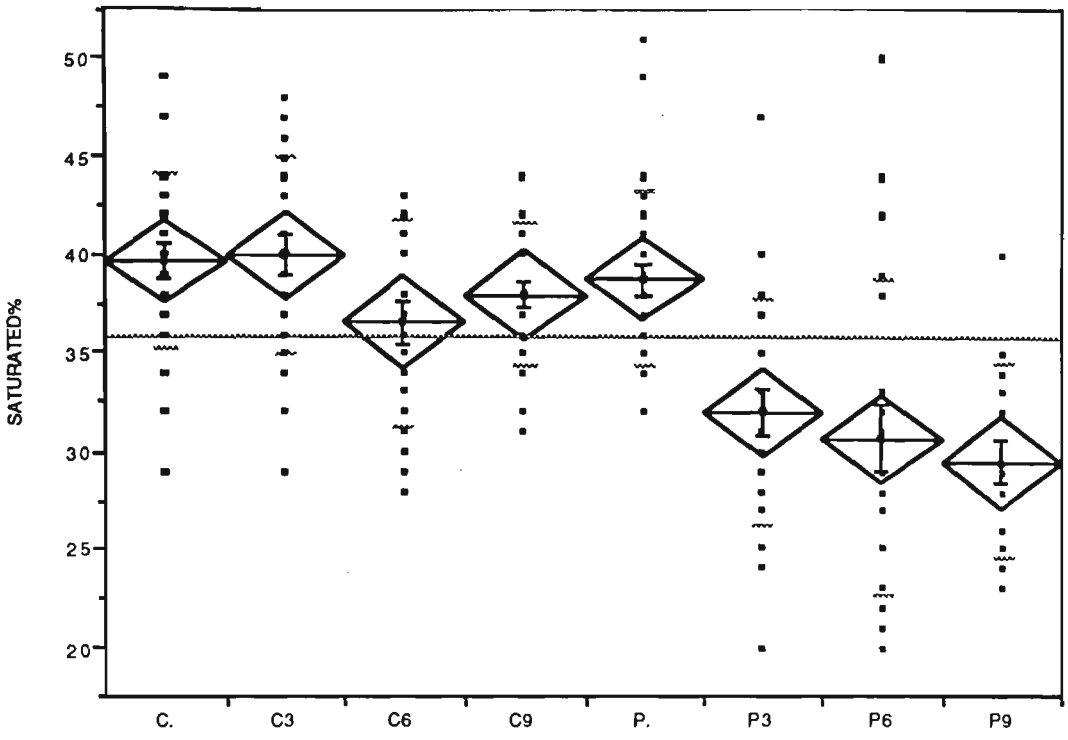

Figure 4.42: Comparison of the mean of saturated fat intake (%)(energy from fat) during the study in HMUFA group (P.=0 time, P3= 3 months, P6= 6 months, P9= 12 months) and Control group (C.=0 time, C3= 3 months, C6= 6 months, C9= 12 months.

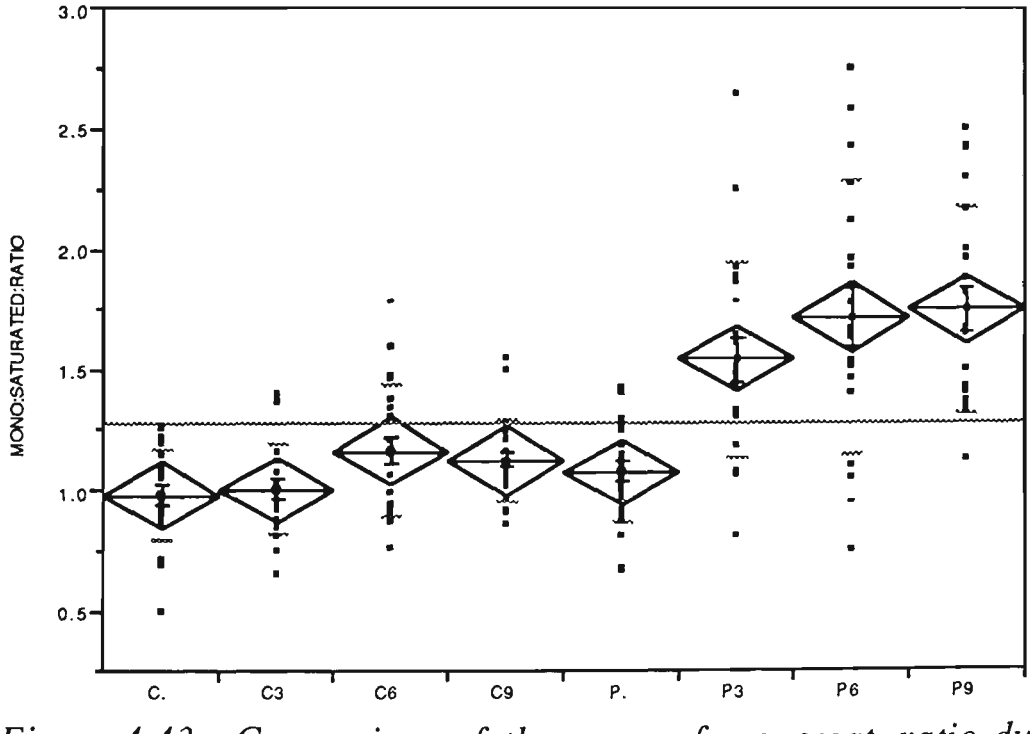

Figure 4.43: Comparison of the mean of mono:sat. ratio during the study in HMUFA group (P.=0 time, P3= 3 months, P6= 6 months, P9= 12 months) and Control group (C.=0 time, C3= 3 months, C6= 6 months, C9= 12 months.

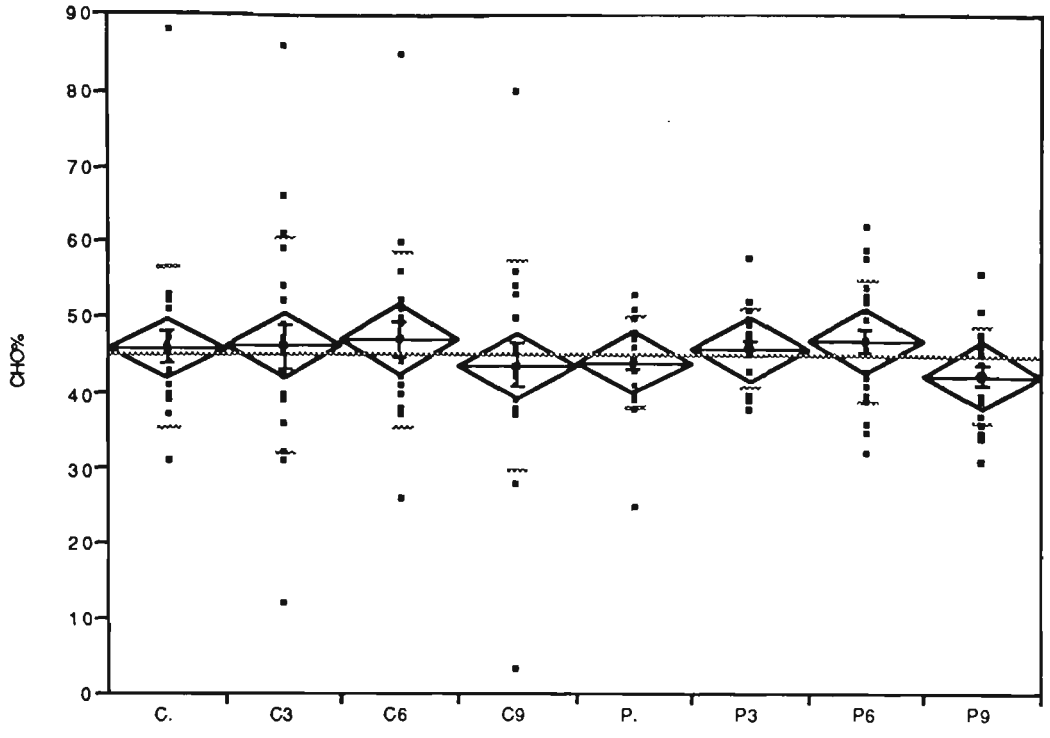

Figure 4.44: Comparison of the mean of carbohydrate intake (%) during the study in HMUFA group (P.=0 time, P3= 3 months, P6= 6 months, P9= 12 months) and Control group (C.=0 time, C3= 3 months, C6= 6 months, C9= 12 months.

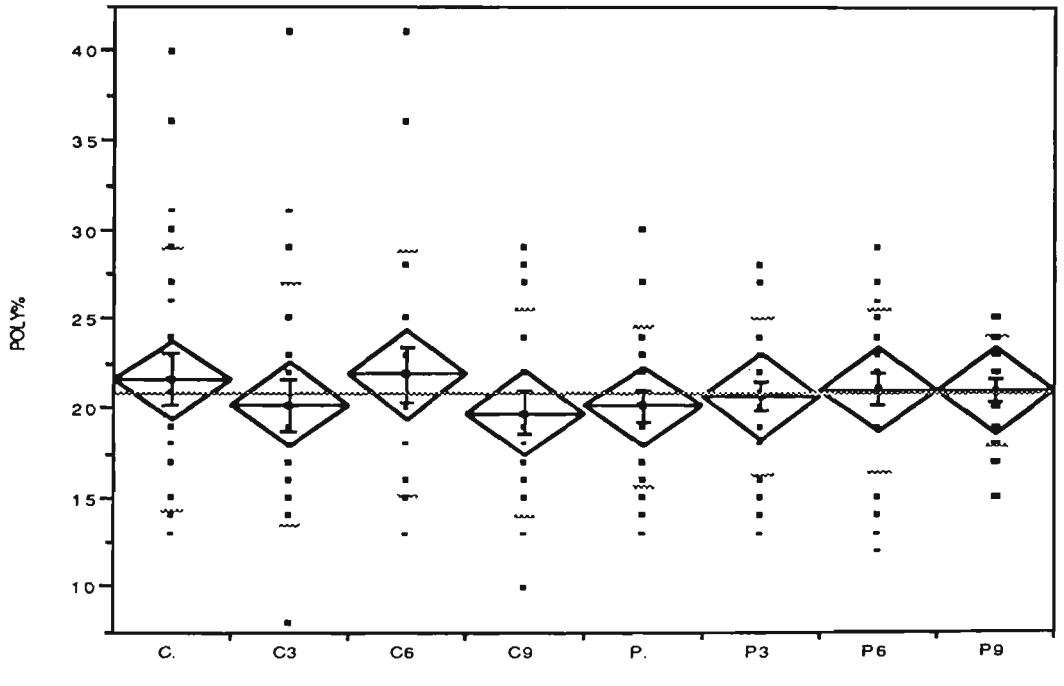

Figure 4.45: Comparison of the mean of polyunsaturated fat intake (%)(energy from fat) during the study in HMUFA group (P.=0 time, P3= 3 months, P6= 6 months, P9= 12 months) and Control group (C.=0 time, C3= 3 months, C6= 6 months, C9= 12 months.

There were significant changes in saturated fat intake (%), monounsaturated fat intake (%), polyunsaturated fat intake (%), monounsaturated:saturated ratio, and polyunsaturated:saturated fat ratio, canola (% and g), and canola n-3 , when the differences between the changes observed at 12 months (compared to base-line) in two diet groups were compared (Table 4.35).

Variable	Control diet 12-0	±SEM	HMUFA diet 12-0	±SEM	Differences: P value
Basic metabolic rate, Kcal	-10	8.5	-3.0	16	0.959
Estimate intake Kcal	68	123	-90.1	110	0.346
Fat, E%	-0.7	1.6	1.4	1.8	0.396
g	-6.4	7.9	0.1	6.4	0.845
CHO, E%	-2.9	2.2	-1.6	1.8	0.734
g	0.1	17	-19.1	14	0.389
Simple CHO%	0.01	0.02	0.001	0.02	0.726
Complex CHO%	0.01	0.02	0.001	0.02	0.726
Dietary fibre, g	-0.3	2.1	-0.7	1.9	0.744
Total sugars, g	-5.7	5.0	-5.7	5.0	0.434
Starch, g	2.9	12.1	-8.5	15.2	0.561
Sat. fat, % total fat	-1.2	1.3	-9	1.2	**0.000***
g	-5.4	4.8	-6.1	2.0	0.155
Poly. fat, % total fat	-2.6	1.4	0.9	1.0	**0.044***
g	-1.8	1.5	1.0	1.5	0.489
Mono. fat, % total fat	3.7	1.2	8.4	1.2	**0.008***
g	-0.8	3.4	4.7	3.1	0.291
Mono:sat:ratio	0.13	0.1	0.66	0.1	**0.000***
Poly:sat. ratio	-0.06	0.05	0.21	0.04	**0.001***
Canola% total fat	2.6	2.2	22.3	3.8	**0.000***
g	1.8	1.1	13.6	4.3	**0.006**
Canola n-3, g	0.2	0.1	1.4	0.4	**0.006**
Total n-6, g	-3.0	1.9	0.43	1.9	0.214
n-6:n-3 ratio	-21.6		-10.65		
Cholesterol mg	-6.4	50	-5.5	24.1	0.523
Protein, E%	1.3	1.9	0.3	0.9	1.000
g	9.5	8.4	-2.2	4.2	0.179
Alcohol, E%	2.1	1.3	-0.4	0.7	0.406
g	7.1	4.7	-1.6	2.9	0.672

Table 4.35: Comparison of differences in daily energy intake in the diet from four days food record between the changes observed in Control diet and HMUFA diet at 12 months (compared to base-line).

In comparison of changes within the group (Table 4.36), there were not any significant changes in Control diet group at 3 months, but there was a significant reduction in sat% intake at 6 and an increase in mono% intake at 12 months. In HMUFA diet group saturated fat intake (% and g) were reduced significantly. Also monounsaturated fat intake (%), mono:sat ratio, poly:sat ratio, canola intake (% and g), and n-3 intake were significantly increased within HMUFA group at 3, 6, and 12 months.

	Control (P value)			HMUFA (P value)		
Variable	**3-0**	**6-0**	**12-0**	**3-0**	**6-0**	**12-0**
Basic metabolic rate, Kcal	0.870	0.839	0.766	0.739	0.773	0.907
Estimate intakeKcal	0.651	0.757	0.887	0.294	0.589	0.531
Fat, E%	0.262	0.343	0.672	0.294	0.381	0.260
g	0.257	0.274	0.399	0.185	0.274	0.344
CHO, E%	0.954	0.752	0.527	0.287	0.161	0.160
g	0.667	0.968	0.955	0.598	0.743	0.912
Simple CHO%	0.692	0.783	0.678	0.842	0.653	0.249
Complex CHO%	0.692	0.783	0.678	0.842	0.653	0.249
Dietary fibre, g	0.783	0.931	0.872	0.834	0.280	0.282
Total sugars, g	0.977	0.881	0.822	0.800	0.176	0.500
Starch, g	0.748	0.982	0.977	0.359	0.316	0.975
Sat. fat, % total fat	0.835	**↓0.036***	0.152	**↓0.000***	**↓0.000***	**↓0.000***
g	0.243	0.125	0.273	**↓0.012***	0.619	0.557
Poly. fat, % total fat	0.485	0.915	0.337	0.672	0.475	0.343
g	0.083	0.423	0.482	0.325	0.606	0.124
Mono. fat, % total fat	0.527	0.116	**↑0.036***	**↑0.000***	**↑0.000***	**↑0.004***
g	0.320	0.462	0.703	0.666	0.972	0.141
Mono:sat:ratio	0.717	**↑0.016***	**↑0.019***	**↑0.000***	**↑0.000***	**↑0.004***
Poly:sat. ratio	0.622	0.439	0.621	**↑0.012***	**↑0.002***	**↑0.006***
Canola% total fat	0.429	0.124	0.303	**↑0.000***	**↑0.000***	**↑0.000***
g	0.718	0.432	0.434	**↑0.040***	**↑0.002***	**↑0.004***
Canola n-3, g	0.178	0.432	0.434	**↑0.040***	**↑0.002***	**↑0.004***
Total n-6, g	0.075	0.364	0.375	0.165	0.844	0.835
n-6:n-3 ratio	0.588	0.393	0.496	0.549	0.174	0.811
Cholesterol, mg	0.759	0.219	0.729	0.443	0.071	0.328
Protein, E%	0.508	0.972	0.538	0.668	0.663	0.840
g	0.969	0.784	0.566	0.487	0.600	0.264
Alcohol, E%	0.087	0.117	0.116	0.697	0.460	0.916
g	0.144	0.141	0.161	0.660	0.498	0.879

*Table 4.36: The P value results of comparison within the group at 3 months (3-0), 6 months (6-0) and 12 months (12-0) in daily energy intake. The significant values are shown with *.*

4.8.2 Minerals and Trace elements:

4.8.2.1 Base-line:

At 0 time, there were no significant differences in calcium, potassium, magnesium, iron, zinc, sodium, or phosphorus intake, between the two diet groups. There were no significant differences in Recommended Daily Intake (RDI) of minerals and trace elements between the two groups (Table 4.37).

Variable	Control diet 0 time	±SEM	HMUFA diet 0 time	±SEM	Differences: P value
Calcium, mg	759	69	741	65	0.856
RDI %	95	9	93	8	0.853
Potassium, mg	3112	220	2941	179	0.547
RDI %	160	11	151	9	0.547
Magnesium, mg	303	24	280	16	0.417
RDI %	100	7	96	5	0.593
Iron, mg	14.4	1.4	12.5	0.7	0.230
RDI %	184	24	140	13	0.108
Zinc, mg	11.7	1.0	10.8	0.6	0.451
RDI %	97.3	8.1	90.3	5	0.456
Sodium, mg	3096	317	2680	173	0.251
Phosphorus, mg	1485	128	1348	73	0.596
RDI %	149	13	135	7	0.355

Table 4.37: Daily intake of minerals and trace elements at base-line (0 time). Mean values and SEM are given. P value refers to comparison between the two groups, using t test.

4.8.2.2 Three months:

There were no significant changes between the Control diet and HMUFA diet at three months time in calcium, potassium, magnesium, iron, zinc, sodium, or phosphorus intake between the two diet groups. (Table 4.38).

Variable	Control diet 3 months	±SEM	HMUFA diet 3 months	±SEM	Differences: P value
Calcium, mg	681	60	735	65	0.547
RDI %	85	7	92	8	0.552
Potassium, mg	3072	215	2871	213	0.511
RDI %	158	11	147	11	0.510
Magnesium, mg	285	22	264	23	0.527
RDI %	94	7	91	8	0.791
Iron, mg	13.6	1.2	11.6	0.9	0.193
RDI %	174	21	130	14	0.087
Zinc, mg	11.8	1.0	9.6	0.9	0.078
RDI %	98	7.9	80	8	0.078
Sodium, mg	2951	285	2321	173	0.066
Phosphorus, mg	1326	93	1190	104	0.336
RDI %	133	9	125	9	0.566

Table 4.38: Daily intake of minerals and trace elements at 3 months. Mean values and SEM are given. P value refers to comparison between the two groups, using t test.

No significant changes were noted in calcium, potassium, magnesium, iron, zinc, sodium, or phosphorus intake when the differences between the changes observed at 3 months (compared to base-line) in two diet groups, were compared (Table 4.39).

Variable	Control diet 3-0	±SEM	HMUFA diet 3-0	±SEM	Differences: P value
Calcium, mg	-69.3	72	-37.2	54.6	0.725
RDI %	-8.7	9.0	-4.7	6.8	1.000
Potassium, mg	-33.3	182	-113	209	0.519
RDI %	-1.8	9.3	-5.9	10.7	0.526
Magnesium, mg	-14	22.2	-20.3	16.6	0.392
RDI %	-5.5	7.6	-5.9	6.2	0.971
Iron, mg	-0.6	1.2	-1.1	0.7	0.139
RDI %	-4.4	17	-15	8	0.051
Zinc, mg	0.4	1.0	-1.3	1.0	0.232
RDI %	3.1	8.0	-11	8.0	0.227
Sodium, mg	-4.6	237	-420	208	0.195
Phosphorus, mg	-129	126	-190.2	90.1	0.124
RDI %	-13	12.7	-13	7.7	0.227

Table 4.39: Comparison of differences in daily intake of minerals and trace elements between the changes observed in Control diet and HMUFA diet at 3 months (compared to base-line).

4.8.2.3 Six months:

There were no significant changes in calcium, potassium, magnesium, iron, zinc, sodium, or phosphorus intake between the Control diet and HMUFA diet at six months time (Table 4.40).

Variable	Control diet 6 months	±SEM	HMUFA diet 6 months	±SEM	Differences: P value
Calcium, mg	782	74	862	96	0.506
RDI %	98	9	105	12	0.612
Potassium, mg	3062	212	3152	136	0.717
RDI %	157	11	162	7	0.720
Magnesium, mg	294	22	298	21	0.895
RDI %	97	6	101	7	0.396
Iron, mg	13.3	1.3	12	0.8	0.835
RDI %	169	22	133	13	0.328
Zinc, mg	10.6	0.9	9.2	0.5	0.156
RDI %	88.5	7.2	77	4.0	0.162
Sodium, mg	2951	286	2527	187	0.430
Phosphorus, mg	1382	119	1347	96	0.895
RDI %	138	12	135	10	0.396

Table 4.40: Daily intake of minerals and trace elements at 6 months. Mean values and SEM are given. P value refers to comparison between the two groups, using t test.

No significant changes were noted in calcium, potassium, magnesium, iron, zinc, sodium, or phosphorus intake when the differences between the changes observed at 3 months (compared to base-line) in two diet groups were compared (Table 4.41).

Variable	Control diet 6-0	±SEM	HMUFA diet 6-0	±SEM	Differences: P value
Calcium, mg	37.4	78	193	192	0.875
RDI %	4.5	10	1.2	13	0.844
Potassium, mg	-66	252	251	275	0.402
RDI %	-3.4	13	12.8	14	0.403
Magnesium, mg	-10.3	22	40.2	30	0.302
RDI %	-3.7	8	134	125	0.296
Iron, mg	-1.1	1.4	0.5	1.4	0.913
RDI %	-17	19	18	25	0.623
Zinc, mg	-0.9	0.9	-1.1	1.0	0.845
RDI %	-7.4	7.6	-9.5	8.2	0.848
Sodium, mg	-148	332	10	319	0.735
Phosphorus, mg	-86	134	83	158	0.418
RDI %	-8.7	13	8.3	16	0.875

Table 4.41: Comparison of differences in daily intake of minerals and trace elements between the changes observed in Control diet and HMUFA diet at 6 months (compared to base-line).

4.8.2.4 Twelve months:

There were no significant changes in calcium, potassium, magnesium, iron, zinc, sodium, or phosphorus intake between the Control diet and HMUFA diet at 12 months time (Table 4.42).

Variable	Control diet 12 months	±SEM	HMUFA diet 12 months	±SEM	Differences: P value
Calcium, mg	761	65	804	50	0.608
RDI %	95	8	101	6	0.599
Potassium, mg	3284	154	3041	161	0.171
RDI %	168	8	156	8	0.279
Magnesium, mg	307	20	295	25	0.309
RDI %	102	6	101	7	0.548
Iron, mg	13.4	1.1	11.5	0.8	0.163
RDI %	163	18	126	14	0.117
Zinc, mg	11.5	1.0	9.4	0.7	0.101
RDI %	96	8.6	79	6	0.148
Sodium, mg	2791	307	2567	206	0.551
Phosphorus, mg	1415	95	1332	97	0.549
RDI %	141	9	133	10	0.566

Table 4.42: Daily intake of minerals and trace elements at 12 months. Mean values and SEM are given. P value refers to comparison between the two groups, using t test.

No significant changes were in calcium, potassium, magnesium, iron, zinc, sodium, or phosphorus intake noted when the differences between the changes observed at 12 months (compared to base-line) in two diet groups were compared (Table 4.43).

Variable	Control diet 12-0	±SEM	HMUFA diet 12-0	±SEM	Differences: P value
Calcium, mg		64	73.7	70	0.279
RDI %	-0.6	8.0	9.3	8.7	0.268
Potassium, mg	132	197	27	183	0.948
RDI %	6.7	10	1.3	9.4	0.969
Magnesium, mg	6.1	23	7.8	17.4	0.705
RDI %	2.0	8.1	2.2	5.9	0.981
Iron, mg	-0.6	1.4	-1.4	0.8	0.211
RDI %	-15.8	9	-16	9	0.328
Zinc, mg	0.4	1.1	-1.7	0.6	0.133
RDI %	3.1	10	-14	5.0	0.128
Sodium, mg	-138	326	-257	319	0.796
Phosphorus, mg	-41.8	130	-36.4	65	0.514
RDI %	-4.3	13	-3.7	6.5	0.969

Table 4.43: Comparison of differences in daily intake of minerals and trace elements between the changes observed in Control diet and HMUFA diet at 12 months (compared to base-line).

In comparison of the mean daily intake of minerals and trace elements differences within the group, there was not any significant changes in Control diet. In HMUFA, there were significant decreases in zinc intake and zinc RDI% at 6 months only (Table 4.44).

	Control (P value)			HMUFA (P value)		
Variable	**3-0**	**6-0**	**12-0**	**3-0**	**6-0**	**12-0**
Calcium, mg	0.403	0.821	0.977	0.944	0.141	0.529
RDI%	0.401	0.830	0.988	0.936	0.372	0.527
Potassium, mg	0.898	0.870	0.531	0.923	0.363	0.357
RDI %	0.894	0.867	0.533	0.926	0.367	0.356
Magnesium, mg	0.577	0.786	0.898	0.966	0.477	0.187
RDI %	0.504	0.698	0.846	0.938	0.273	0.229
Iron, mg	0.655	0.554	0.561	0.930	0.643	0.619
RDI %	0.765	0.653	0.491	0.885	0.712	0.375
Zinc, mg	0.955	0.425	0.920	0.547	**↓0.044***	0.782
RDI %	0.948	0.422	0.929	0.544	**↓0.043***	0.784
Sodium, mg	0.737	0.493	0.496	0.506	0.551	0.700

*Table 4.44: The P value results of comparison within the group at 3 months (3-0), 6 months (6-0) and 12 months (12-0) in daily intake of minerals and trace elements. The significant values are shown with *.*

4.8.3 Vitamins:

4.8.3.1 Base-line:

At 0 time, there were no significant differences in thiamine, riboflavin, niacin, total A, and vitamin C intake, between the two diet groups. There were no significant differences in Recommended Daily Intake (RDI) of vitamins between the two groups (Table 4.45).

Variable	Control diet 0 time	±SEM	HMUFA diet 0 time	±SEM	Differences: P value
Thiamine, mg	1.34	0.12	1.38	0.1	0.821
Rdi%	130%	11	132%	8	0.903
Riboflavin, mg	1.89	0.2	1.78	0.1	0.648
Rdi%	124%	13	114%	7	0.518
Niacin Eq, mg	39.2	3.2	38.8	2.0	0.913
Rdi%	234%	16	232%	10	0.897
Total A Eq, ug	1446	408	1011	116	0.882
Rdi%	193%	54	135%	15	0.857
Vitamin C, mg	167	40	117	14	0.807
Rdi%	486%	127	361%	53	0.958

Table 4.45: Daily intake of vitamins at base-line (0 time). Mean values and SEM are given. P value refers to comparison between the two groups, using t test.

4.8.3.2 Three months:

There were no significant differences in thiamine, riboflavin, niacin, total A, and vitamin C intake, between the Control diet and HMUFA diet at three months time (Table 4.46).

Variable	Control diet 3 months	±SEM	HMUFA diet 3 months	±SEM	Differences: P value
Thiamine, mg	1.37	0.1	1.41	0.1	0.841
Rdi%	136%	14.8	133%	11	0.882
Riboflavin, mg	1.76	0.2	1.71	0.1	0.803
Rdi%	117%	13	109%	9	0.604
Niacin Eq, mg	41.3	3.3	36.3	2.6	0.243
Rdi%	246%	17	215	15	0.171
Total A Eq, ug	1232	190	1045	144	0.550
Rdi%	164%	25	139%	19	0.526
Vitamin C, mg	134	26	101	14	0.769
Rdi%	380%	82	304%	34	0.981

Table 4.46: Daily intake of vitamins at 3 months. Mean values and SEM are given. P value refers to comparison between the two groups, using t test.

No significant changes noted when the differences in thiamine, riboflavin, niacin, total A, and vitamin C intake, between the changes observed at 3 months (compared to base-line) in two diet groups were compared, except the significant decrease of niacin intake in HMUFA group (Table 4.47).

Variable	Control diet 3-0	±SEM	HMUFA diet 3-0	±SEM	Differences: P value
Thiamine, mg	0.05	1.4	-0.005	0.1	0.758
Rdi%	6.1	14	-0.8	10	0.697
Riboflavin, mg	-0.1	0.2	-0.1	0.1	0.541
Rdi%	-7.2	14	-7.5	8	0.760
Niacin Eq, mg	2.9	2.6	-3.1	2.0	**0.012***
Rdi%	14.7	15	-17.7	13	0.113
Total A Eq, ug	111	143	31	92	0.743
Rdi%	14.8	19	4.0	12	0.742
Vitamin C, mg	-114	96	-11	15	0.707
Rdi%	-35	34	-36	46	0.751

Table 4.47: Comparison of differences in daily intake of vitamins between the changes observed in Control diet and HMUFA diet at 3 months (compared to base-line).

4.8.3.3 Six months:

There were no significant changes in thiamine, riboflavin, niacin, total A, and vitamin C intake, between the Control diet and HMUFA diet at six months time (Table 4.48).

Variable	Control diet 6 months	±SEM	HMUFA diet 6 months	±SEM	Differences: P value
Thiamine, mg	1.56	0.2	1.76	0.2	0.413
Rdi%	153%	14	170%	18	0.460
Riboflavin, mg	2.01	0.2	2.07	0.2	0.822
Rdi%	132%	14	133%	14	0.971
Niacin Eq, mg	40.4	3.2	39.4	2.6	0.807
Rdi%	241%	16	227%	19	0.938
Total A Eq, ug	927	154	951	147	0.411
Rdi%	124%	21	140%	18	0.106
Vitamin C, mg	167	37	108	15	0.188
Rdi%	479%	121	324%	51	0.355

Table 4.48: Daily intake of vitamins at 6 months. Mean values and SEM are given. P value refers to comparison between the two groups, using t test.

No significant changes in thiamine, riboflavin, niacin, total A, and vitamin C intake, noted when the differences between the changes observed at 6 months (compared to base-line) in two diet groups were compared (Table 4.49).

Variable	Control diet 6-0	±SEM	HMUFA diet 6-0	±SEM	Differences: P value
Thiamine, mg	0.3	0.2	0.4	0.2	0.721
Rdi%	29	18	29	16	0.971
Riboflavin, mg	0.2	0.3	0.2	0.2	0.817
Rdi%	13	19	10	14	0.489
Niacin Eq, mg	1.8	3.1	3.3	3.5	0.952
Rdi%	13	19	-0.2	21	0.647
Total A Eq, ug	-480	480	-84	171	0.961
Rdi%	13	19	-0.2	21	0.761
Vitamin C, mg	-7.6	53	0.05	26	0.568
Rdi%	-29	172	-32	80	0.697

Table 4.49: Comparison of differences in daily intake of vitamins between the changes observed in Control diet and HMUFA diet at 6 months (compared to base-line).

4.8.3.4 Twelve months:

There were no significant changes in thiamine, riboflavin, niacin, total A, and vitamin C intake, between the Control diet and HMUFA diet at 12 months time (Table 4.50).

Variable	Control diet 12 months	±SEM	HMUFA diet 12 months	±SEM	Differences: P value
Thiamine, mg	1.47	0.1	1.46	0.1	0.945
Rdi%	142%	11	140%	11	0.896
Riboflavin, mg	1.81	0.1	1.83	0.1	0.937
Rdi%	119%	10	118%	10	0.939
Niacin Eq, mg	44.9	4.0	38.6	3.1	0.223
Rdi%	264%	16	230%	15	0.081
Total A Eq, ug	846	113	948	111	0.522
Rdi%	113%	15	126%	15	0.303
Vitamin C, mg	148	30	101	15	0.230
Rdi%	418%	89	311%	46	0.375

Table 4.50: Daily intake of vitamins at 12 months. Mean values and SEM are given. P value refers to comparison between the two groups, using t test.

No significant changes noted in thiamine, riboflavin, niacin, total A, and vitamin C intake, when the differences between the changes observed at 12 months (compared to base-line) in two diet groups were compared, except the changes in niacin intake (Table 4.51).

Variable	Control diet 12-0	±SEM	HMUFA diet 12-0	±SEM	Differences: P value
Thiamine, mg	0.11	0.1	0.03	0.1	0.646
Rdi%	9.3	13.6	4.5	13	0.797
Riboflavin, mg	-0.07	0.2	-0.005	0.1	0.715
Rdi%	-5.7	13	1.3	10	0.896
Niacin Eq, mg	6.7	3.3	-1.5	2.0	**0.009***
Rdi%	33.2	17	-8.8	13	0.062
Total A Eq, ug	-97	91	-86	101	0.818
Rdi%	-13	12	-11.6	14	0.829
Vitamin C, mg	-97	96	-21	16	0.557
Rdi%	-27	31	-70	50	0.611

Table 4.51: Comparison of differences in daily intake of vitamins between the changes observed in Control diet and HMUFA diet at 12 months (compared to base-line).

In comparison of the differences within the group, there were no significant changes at 3, 6 , or 12 months in either group (Table 4.52).

	Control (P value)			HMUFA (P value)		
Variable	3-0	6-0	12-0	3-0	6-0	12-0
Thiamine, mg	0.872	0.264	0.492	0.644	0.069	0.377
Rdi%	0.757	0.215	0.451	0.740	0.057	0.615
Riboflavin, mg	0.623	0.665	0.746	0.825	0.250	0.797
Rdi%	0.708	0.660	0.781	0.707	0.231	0.982
Niacin Eq, mg	0.654	0.799	0.271	0.722	0.862	0.379
Rdi%	0.613	0.754	0.199	0.565	0.815	0.620
Total A Eq, ug	0.643	0.267	0.180	0.807	0.747	0.281
Rdi%	0.643	0.266	0.181	0.802	0.811	0.281
Vitamin C, mg	0.493	0.994	0.718	0.367	0.702	0.967
Rdi%	0.488	0.967	0.664	0.296	0.614	0.745

*Table 4.52: The P value results of comparison with baseline values within each group at 3 months (3-0), 6 months (6-0) and 12 months (12-0) in daily intake of vitamins. The significant values are shown with *.*

4.8.4 Diet acceptability:

Diet acceptability was measured using questionnaire which the participants completed at 12 months time (Appendix II). The questionnaire had 8 questions with Lichert Scale of zero to ten (0-10). The HMUFA group felt that their diet is easier to prepare (P=0.010*), is tastier (P=0.028*), it makes them more satisfied (P=0.036*), and is more convenient for their family (P=0.002).

There were no significant differences in the cost of the food, variety, or adherence to the diet between Control and HMUFA diet (Table 4.53).

A selection of general comments on dietary difficulties which were given by participants are as follows:

Control diet group:

* "I have three teenagers children, they are always hungry, and since my wife comes home at 6 pm so I have to cook for them and that makes it difficult to adhere to my own diet."
* "My only problem is beer."
* "My problem is vegetables, because the rest of the family don't like it."
* "I am still hungry after the dinner."
* "Sticking to diet when `cheating food' is in the house, is very difficult."
* "This diet is very boring, that's why I often break out and do the wrong things."
* "I wish more restaurant had diabetic diet."
* "Since I am the only diabetic in the family, this diet is not convenient for them, therefore is difficult for me to follow."
* "Usually after 2 weeks of following the diet I get very bored and start eating the wrong foods."
* "In cold weather I always want some fatty food therefore I break the diet."
* "I am happy with this diet, only I have problem with my family, because they don't like these sort of foods."

Two terms that became apparent were that participant's families had, in general, not taken up a High-CHO low fat diet pattern for themselves, and participants found this diet pattern "boring" and difficult to persist with.

HMUFA diet group:

* "This is a good diet, considering the catering for four children."
* "Although I didn't lost weight, but my blood sugar is more stable and I feel much healthier since I started this diet."
* "Preparing the food and finding the food was very easy, but I didn't stick to it as much as I should."
* "I had no problem with the diet, only I think I had too much of good food and no exercises."
* "I prefer this diet to the other one because is easier to follow."
* " In the beginning I wasn't sure if I should have that much fat in my diet, but later on I noted that my blood sugar was more stable and I haven't had any 'cold' this year."
* "Will power and motivation are my problem rather than the diet."
* "I had problem with my wife in the beginning of the program because she didn't believed in having nuts and avocado in the diet."
* "Not only I benefited from this diet, all my family were also happy with this and I tell to my friends about it as well, because I feel much healthier now and I haven't been sick this year at all."
* "In the beginning not eating sausages and red meat was very difficult. It took me some months to reduce it to once a week."

The HMUFA diet pattern seemed more readily accepted by the families or spouses of the participants, and they did not complain of "boredom" or (generally speaking) difficulty in persisting with it.

Variable	Control	±SEM	HMUFA	±SEM	Differences: P value
Ease of preparation%	70	0.5	86	0.4	**0.009***
Cost %	60	0.5	60	0.5	0.971
Taste %	60	0.7	80	0.4	**0.028***
Satisfy %	68	0.6	84	0.4	**0.036***
Variety %	70	0.6	78	0.4	0.468
Adherence %	67	0.5	65	0.5	0.754
Convenient for the family %	60	0.7	87	0.4	**0.002***

Table 4.53: Results of diet acceptability at 12 months following Control diet or HMUFA diet.

4.9 Sub-groups:

A comparison of the sub-groups who underwent an estimation of insulin sensitivity by the euglycemic clamp technique indicated that the differences observed in the two diet groups were found also in the sub-groups.

4.9.1 Euglycemic Clamp sub-group:

4.9.1.1 Base-line:

At base-line (0 time), there were no significant differences between Control diet and HMUFA diet groups, in anthropometric measurements, lipid and lipoprotein measurements, indices of glucose metabolism, past history, medication intake, indices of insulin sensitivity obtained by the euglycemic clamp technique, or diet intake (data not shown).

4.9.1.2 Three months:

At 3 months time also there were no significant differences between Control diet and HMUFA diet groups, in anthropometric measurements, lipid and lipoprotein measurements, indices of glucose metabolism, past history, or medication intake. But there were significant differences in diet intake (Table 4.54, and Figures 4.46 to 4.49).

Variable	Control diet 3 months	±SEM	HMUFA diet 3 months	±SEM	Differences: P value
Monounsaturated fat % (E)	41.3	1.2	47.9	1.2	**0.001***
Saturated fat intake % (E)	39.8	1.3	31.8	1.6	**0.001***
Mono:Sat ratio	1.1	0.1	1.6	0.1	**0.002***
Cholesterol mg	270	29	199	18	**0.039***

Table 4.54: The mean ± SEM daily energy intake in the diet according to the mean of four days food record in clamp study sub-group at 3 months.

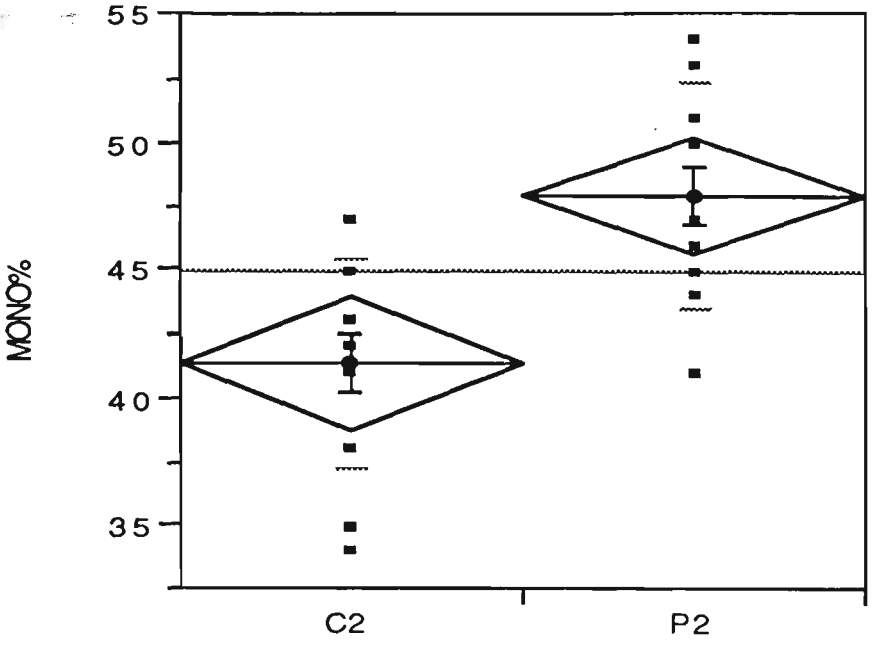

Figure 4.46: Comparison of the mean ± SEM of monounsaturated intake% at 3 months time between Control (C2) and HMUFA (P2) clamp study sub-group (P=0.001).*

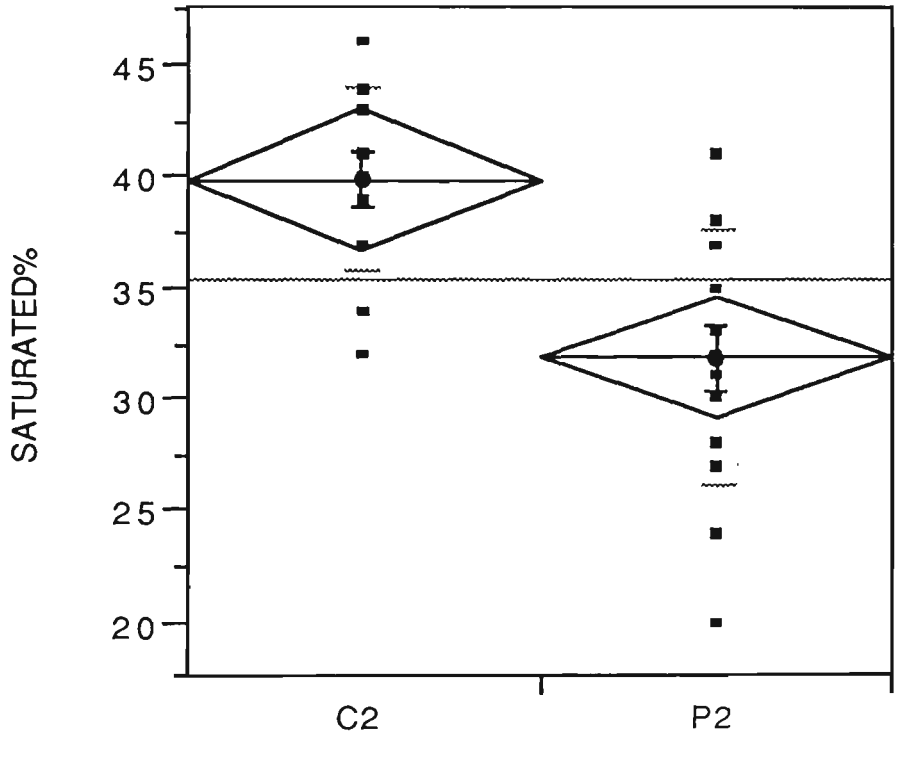

Figure 4.47: Comparison of the mean ± SEM of saturated intake% at 3 months time between Control (C2) and HMUFA (P2) clamp study sub-group (P=0.001).*

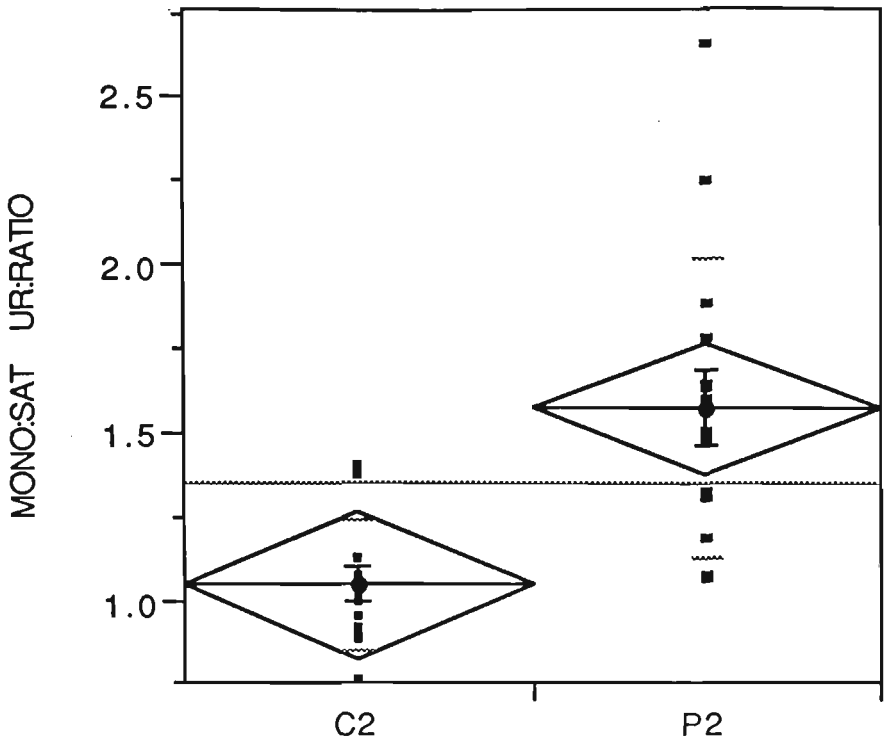

Figure 4.48: Comparison of the mean ± SEM of mono:sat ratio at 3 months time between Control (C2) and HMUFA (P2) clamp study sub-group (P=0.002).*

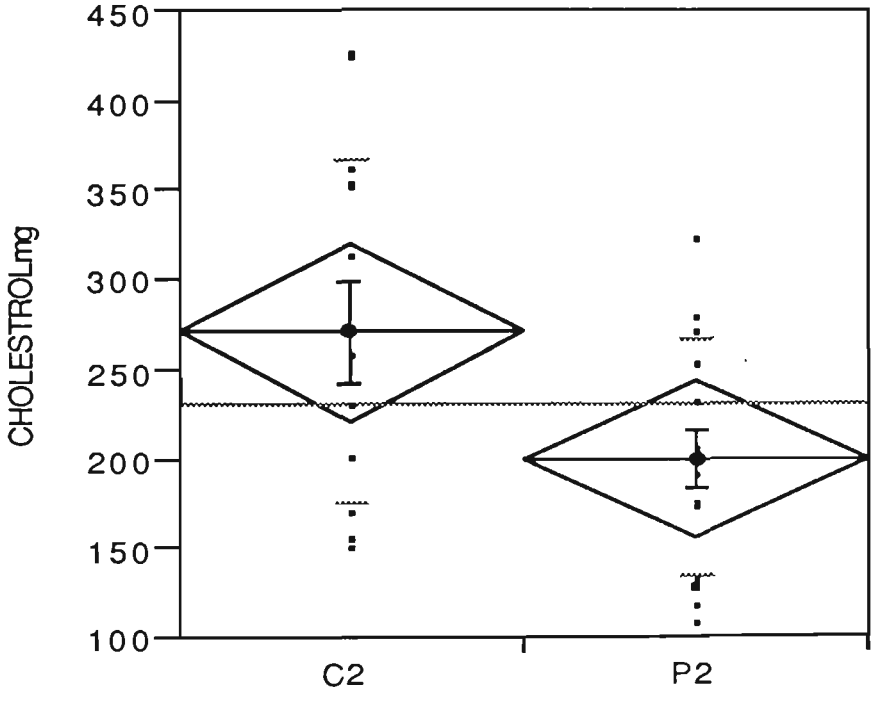

Figure 4.49: Comparison of the mean ± SEM of cholesterol intake in the diet at 3 months time between Control (C2) and HMUFA (P2) in clamp study sub-group (P=0.039).*

There were also significant changes in waist, hip, biceps, triceps, suprailiac skinfold thickness, skinfold sum, body fat % measurements, plasma apo A, saturated fat intake (%), mono:sat ratio, and poly:sat ratio when the differences between the changes observed at 3 months (compared to base-line) in two diet groups were compared (Table 4.55).

Variable	Control diet 3-0	±SEM	HMUFA diet 3-0	±SEM	Differences: P value
Waist (cm)	-2.0	1.1	-5.8	0.8	**0.002***
Hip (cm)	0.2	1.1	-3.1	1.0	**0.039***
Triceps (mm)	1.96	1.5	-8.1	3.0	**0.007***
Suprailiac (mm)	-4.2	3.4	-9.6	2.1	**0.045***
Skinfold sum (mm)	-4.1	3.4	-9.6	2.1	**0.048***
Body fat %	0.3	0.9	-4.0	0.8	**0.001***
P.Apo A (g/L)	0.11	0.06	-0.08	0.06	**0.028***
Saturated F. % total fat	-0.7	2.4	-8.3	1.8	**0.019***
Mono:sat ratio	0.07	0.1	0.52	0.1	**0.011***
Poly:sat ratio	-0.04	0.1	0.22	0.1	**0.028***

Table 4.55: Comparison of differences in diet intake, anthropometric and lipid measurement between the changes observed in Control diet and HMUFA diet clamp study sub-group at 3 months (compared to base-line).

4.9.1.3 Six months:

At six months time only the diet intake was analysed. There were significant differences in monounsaturated fat (%) intake (Control: 40.8± 2.1, High-MUFA: 48.2± 2.1, P=0.006*), and mono:sat ratio (Control: 1.17± 0.1, High-MUFA: 1.68± 0.1, P=0.013*) between the two sub-groups. There were also significant

changes in monounsaturated fat (%) intake (Control: 2.0± 1.8, High-MUFA: 7.5± 1.4, P=0.024*), and mono:sat ratio (Control: 0.19±0.1, High-MUFA: 0.63± 0.1, P=0.016) when the differences between the changes observed at 6 months (compared to base-line) in two diet groups were compared.

4.9.1.4 Twelve months:

At twelve months time there were significant changes in anthropometric measurements, lipid and lipoproteins measurements, glucose uptake, and diet intake between the two sub-groups (Table 4.56, Figures 4.50 to 4.59).

Variable	Control diet 12 months	±SEM	HMUFA diet 12 months	±SEM	Differences: P value
Weight (kg)	84.4	3.7	98.5	2.9	**0.006***
Diastolic B.P mmHg	80	2.6	72.7	1.7	**0.022***
NEFA mmol/L	0.78	0.1	0.39	0.03	**0.003***
VLDL-C mmol/L	0.97	0.1	0.63	0.1	**0.015***
VLDL-TG mmol/L	1.40	0.3	0.68	0.1	**0.017***
IDL-C mmol/L	0.11	0.02	0.05	0.01	**0.018***
HDL-C mmol/L	0.75	0.07	0.98	0.04	**0.004***
LDL:HDL ratio	4.1	0.4	2.9	0.2	**0.001***
CETP (%)	51.4	1.6	27.4	1.1	**0.000***

Table 4.56: Comparison of the mean ± SEM of anthropometric and lipid measurement significant differences between HMUFA diet and Control diet clamp study sub-group at 12 months.

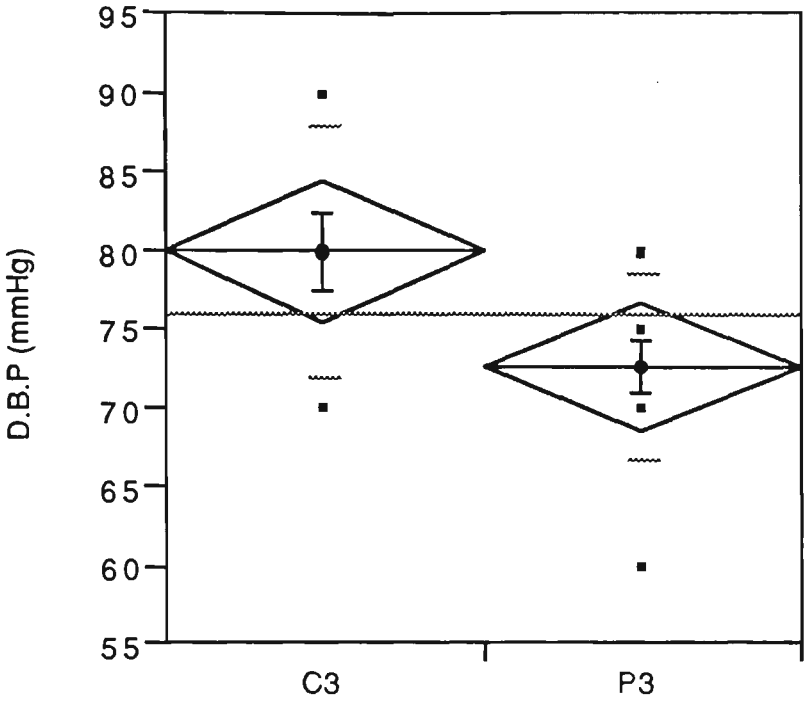

Figure 4.50: Comparison of the mean ± SEM of diastolic blood pressure in Control (C3) and HMUFA (P3) clamp study sub-group at 12 months (P=0.022).*

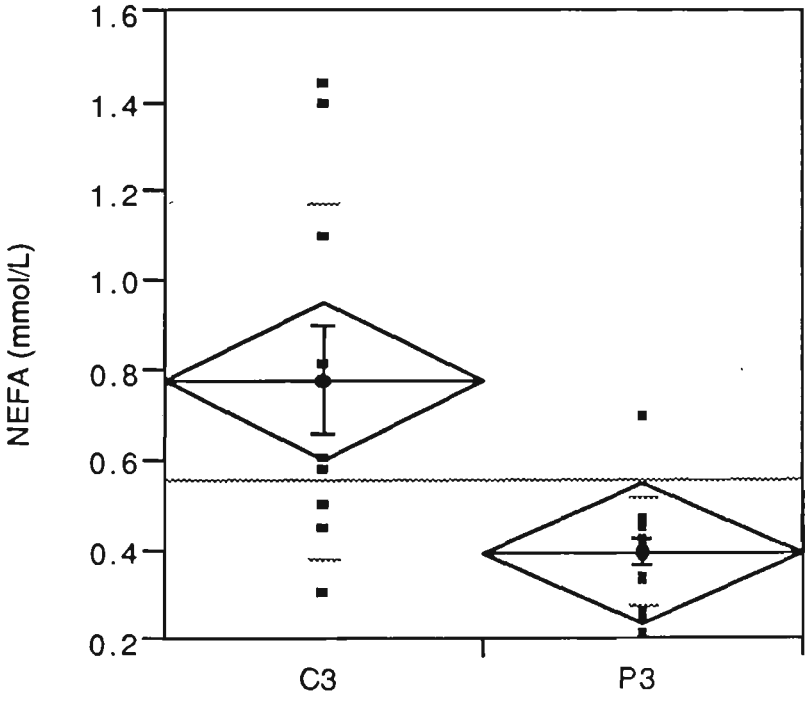

Figure 4.51: Comparison of the mean ± SEM of NEFA in Control (C3) and HMUFA (P3) clamp study sub-group at 12 months (P=0.003).*

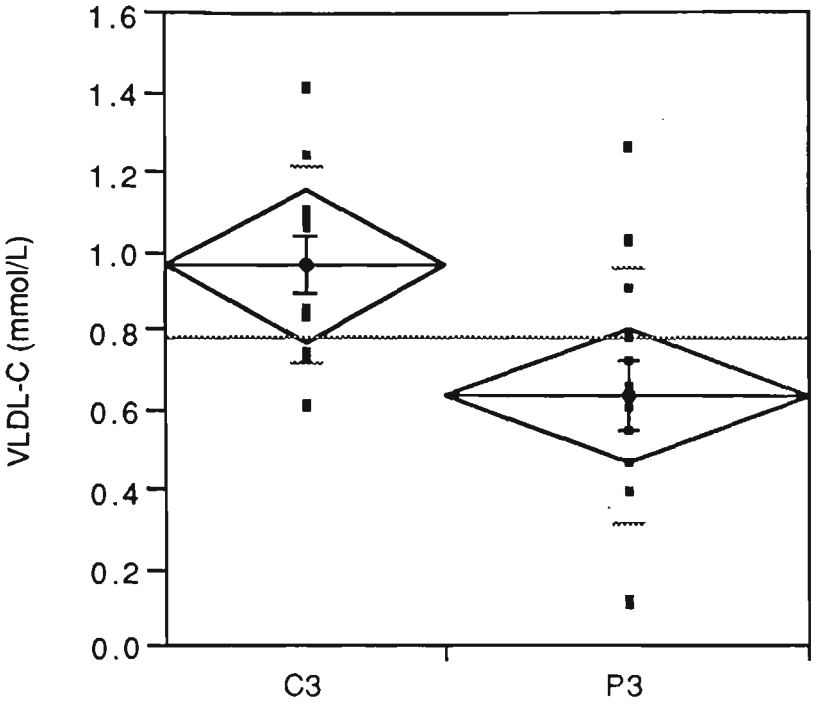

Figure 4.52: Comparison of the mean ± SEM of VLDL-C in Control (C3) and HMUFA (P3) clamp study sub-group at 12 months (P=0.015).*

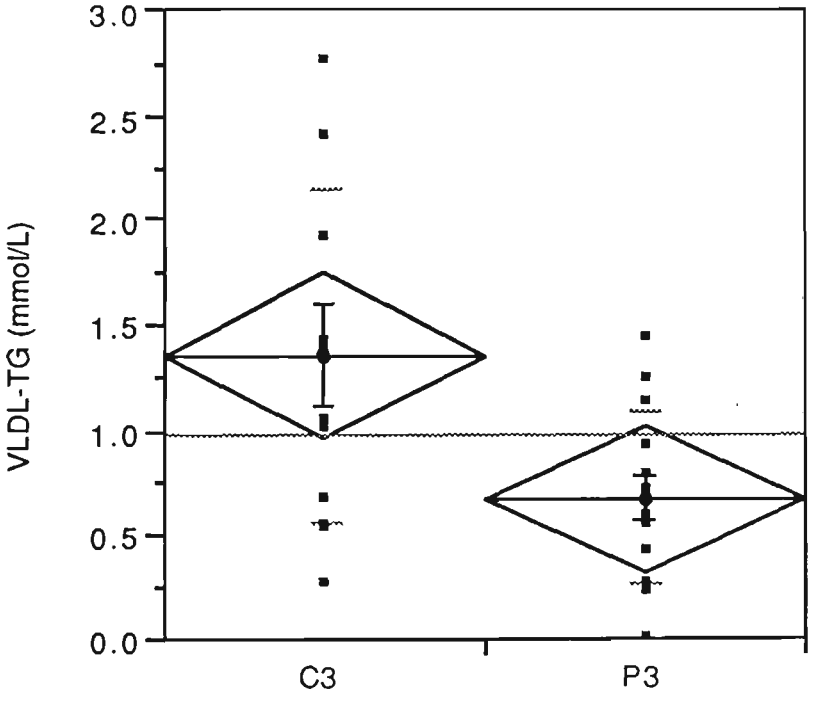

Figure 4.53: Comparison of the mean ± SEM of VLDL-TG in Control (C3) and HMUFA (P3) clamp study sub-group at 12 months (P=0.017).*

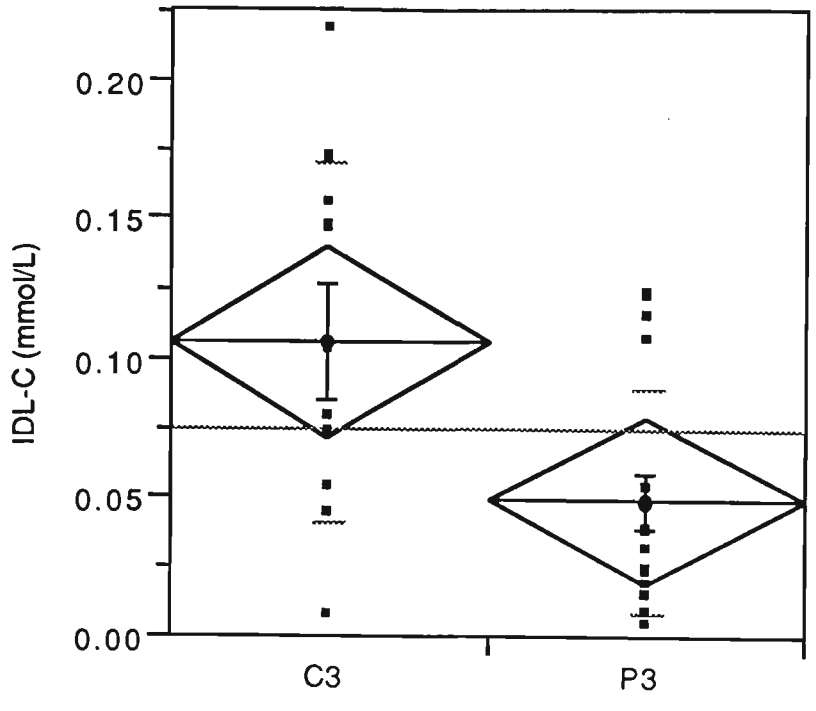

Figure 4.54: Comparison of the mean ± SEM of IDL-C in Control (C3) and HMUFA (P3) clamp study sub-group at 12 months (P=0.018).*

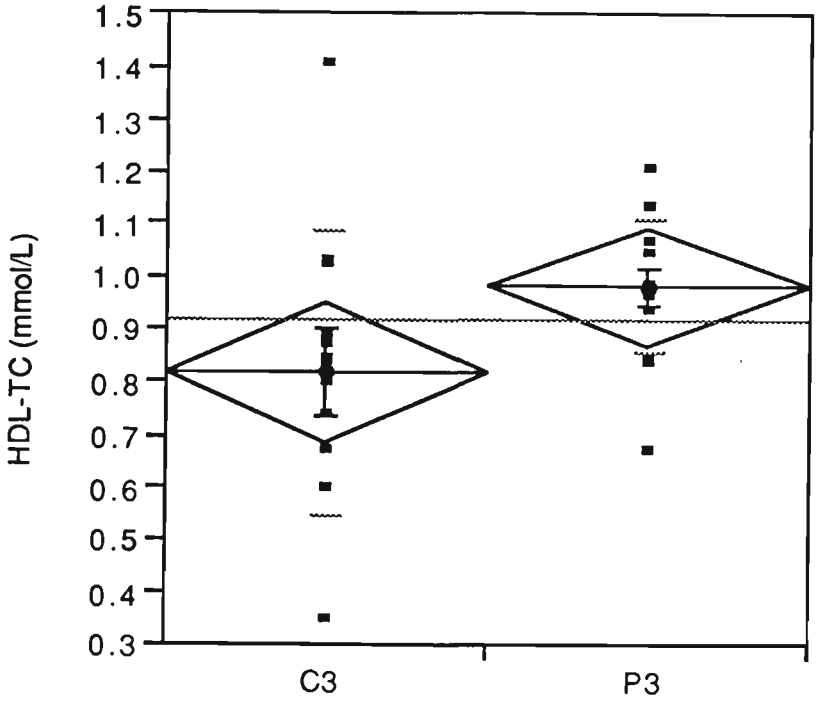

Figure 4.55: Comparison of the mean ± SEM of HDL-TC in Control (C3) and HMUFA (P3) clamp study sub-group at 12 months (P=0.004).*

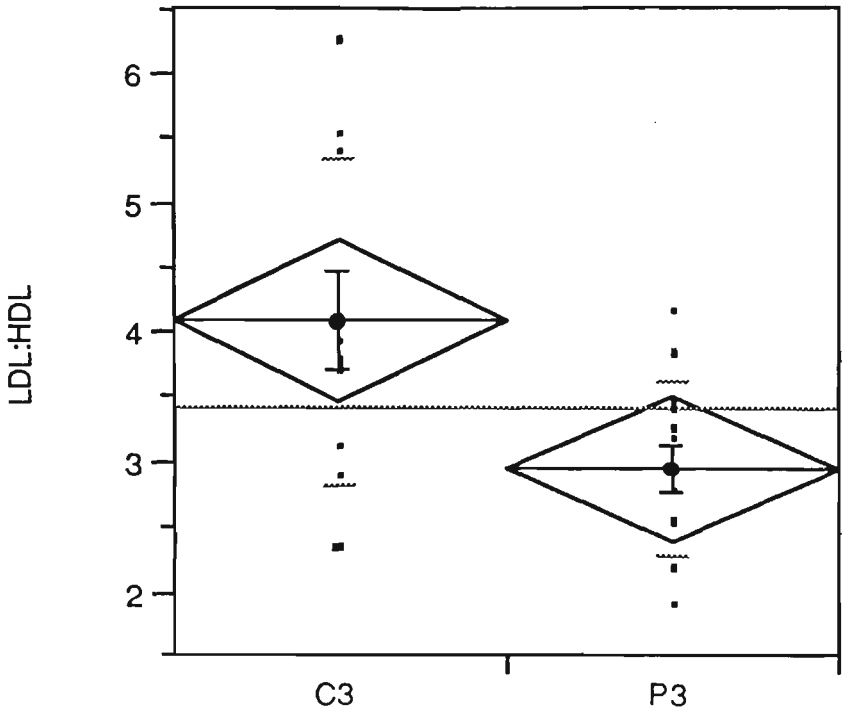

Figure 4.56: Comparison of the mean ± SEM of LDL:HDL ratio in Control (C3) and HMUFA (P3) clamp study sub-group at 12 months (P=0.001).*

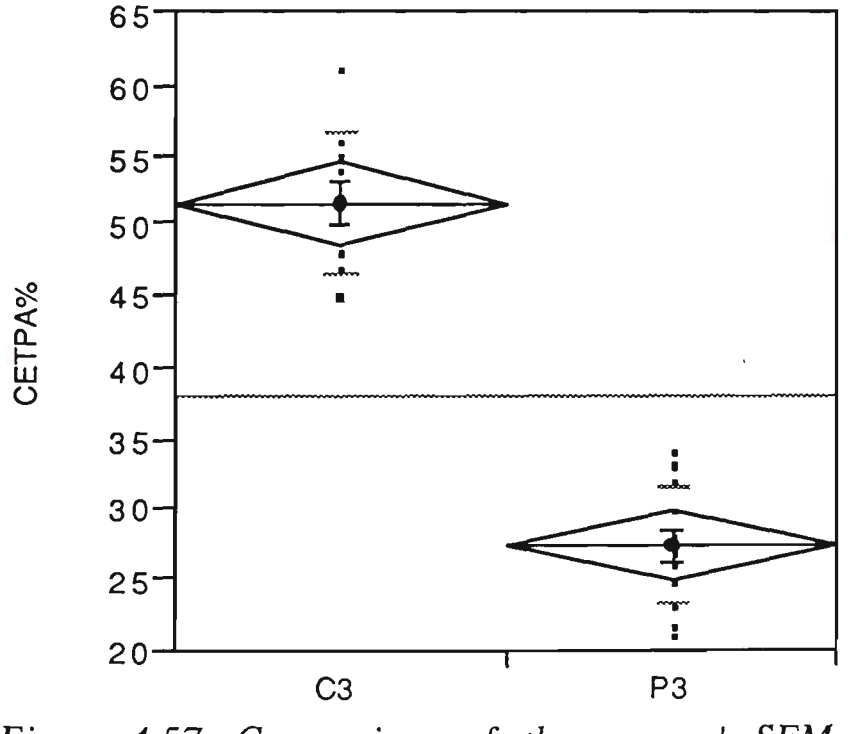

Figure 4.57: Comparison of the mean ± SEM of CETP(%) in Control (C3) and HMUFA (P3) clamp study sub-group at 12 months (P=0.000).*

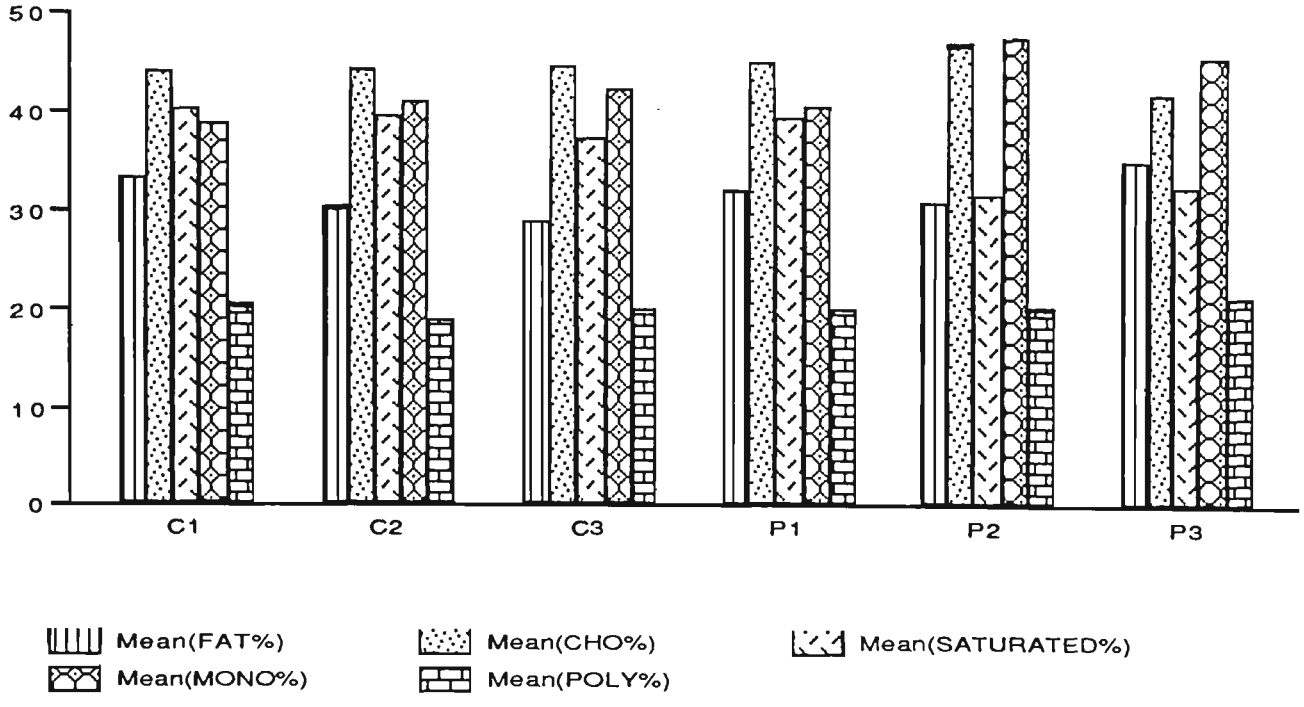

Figure 4.58: Comparison of the mean ± SEM of fat intake %, CHO%, monounsaturated fat (%) intake, saturated fat (%) intake, and polyunsaturated fat (%) intake during 12 months in the sub-group of clamp study in Control diet (C.-C3-C9) and HMUFA diet (P.-P3-P9).
C.&P.= 0 time, C3&P3= 3 months, C6& C9&P9= 12 months.

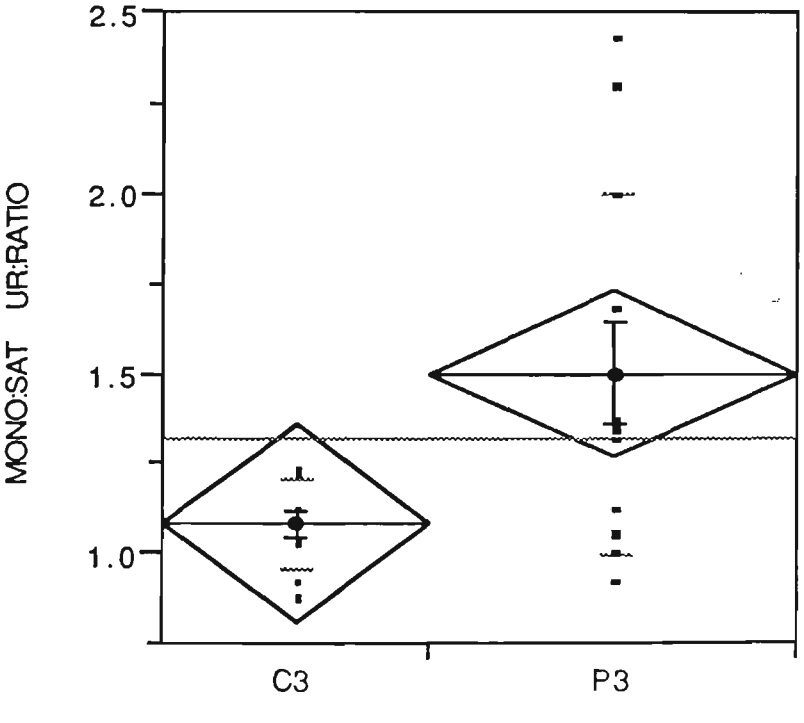

Figure 4.59: Comparison of the mean ± SEM of mono:sat ratio in Control (C3) and HMUFA (P3) clamp study sub-group at 12 months (P=0.000).*

There were also significant changes in triceps skinfold thickness, skinfold sum, body fat%, FBG, plasma apo A, NEFA, HDL-apo A, CETP%, saturated fat intake (%), monounsaturated fat (%) intake, mono:sat ratio, and poly:sat ratio when the differences between the changes observed at 12 months (compared to base-line) in two diet groups were compared (Table 4.57).

Variable	Control diet 12-0	±SEM	HMUFA diet 12-0	±SEM	Differences: P value
Triceps	1.96	1.5	-8.1	3.0	**0.007***
Skinfold sum mm	5.6	6.6	-25.5	7.5	**0.005***
Fat intake % total fat	1.1	0.7	-5.4	1.7	**0.002***
FBG mmol/L	1.5	0.5	-0.5	0.4	**0.007***
P.Apo A g/L	0.12	0.09	-0.13	0.06	**0.021***
NEFA mmol/L	0.35	0.1	-0.06	0.06	**0.006***
HDL-Apo A g/L	0.02	0.07	0.23	0.07	**0.037***
CETP%	14.9	3.2	-7.1	1.9	**0.001***
Saturated F. % total fat	-1.8	2.1	-10.8	1.4	**0.001***
Monounsaturated F % total fat	3.5	1.1	8.9	1.2	**0.004***
Mono:sat:ratio	0.1	0.1	0.8	0.1	**0.000***
Poly:sat. ratio	-0.03	0.1	0.3	0.1	**0.002***

Table 4.57: Comparison of differences in diet intake, FBG, anthropometric and lipid measurement between the changes observed in Control diet and HMUFA diet clamp study sub-group at 12 months (compared to base-line).

In comparison of changes within the group, there were significant changes in anthropometric, lipid and lipoprotein in both sub-groups at 3 months and 12 months (Table 4.58).

	Control (P value)		HMUFA (P value)	
Variable	3-0	12-0	3-0	12-0
Diastolic B.P mmHg	0.222	0.596	0.173	↓0.003*
MAP mmHg	0.703	1.000	0.158	↓0.011*
Suprailiac mm	0.416	0.523	↓0.007*	0.095
Skinfold sum mm	0.975	0.651	↓0.013*	0.085
IDL-C mmol/L	0.534	0.978	0.597	↓0.008*
IDL-TG mmol/L	0.326	0.625	0.206	↓0.023*
HDL-Apo A g/L	0.071	0.635	↑0.028*	↑0.009*
NEFA mmol/L	0.567	0.010*	0.638	0.212
CETP (%)	↓0.028*	↑0.000*	0.506	↓0.002*

*Table 4.58: The P value results of comparison within the group at 3 months (3-0), and 12 months (12-0) in anthropometric, lipid and lipoprotein measurement. The significant values are shown with *.*

4.9.2 Male sub-group:

In order to examine the possibility that the responses were different between sexes, we undertook separate analyses of men and women.

4.9.2.1 Base-line:

At 0 time (Base-line), there were no significant differences between Control diet and HMUFA diet male sub-group, in anthropometric measurements, lipid and lipoproteins measurements, indices of glucose metabolism, past history, medication intake, indices of insulin sensitivity obtained by the euglycemic clamp technique, or diet intake.

4.9.2.2 Three months:

At 3 months time also there were no significant differences between Control diet and HMUFA diet, in anthropometric measurements, lipid and lipoproteins measurements, indices of glucose metabolism, past history, glucose uptake, or medication intake. But there were significant differences in diet intake (Table 4.59).

Variable	Control diet 3 months	±SEM	HMUFA diet 3 months	±SEM	Differences: P value
Estimate intake	2168	141	1753	77	**0.031***
Monounsat.F % total fat	41	1.2	48	1.4	**0.001***
Saturated.F % total fat	42	1.1	31	1.7	**0.000***
Mono:Sat:Ratio	0.99	0.04	1.6	0.1	**0.000***
Poly:sat. ratio	0.44	0.04	0.70	0.08	**0.004***
Protein.g	115	7.2	94.4	3.4	**0.032***
Sodium	3636	270	2740	219	**0.025***
Fat.g	37.5	4.8	57.1	4.0	**0.006***
Saturated.Fat.g	29.5	2.2	16.1	1.6	**0.000***

Table 4.59: Comparison of the mean ± SEM of diet intake between HMUFA diet and Control diet in male sub-group, at 3 months (only significant differences are shown).

There were significant changes in anthropometric and lipoprotein measurements and diet intake when the differences between the changes observed at 3 months (compared to base-line) in the two male sub-groups were compared (Table 4.60).

Variable	Control diet 3-0	±SEM	HMUFA diet 3-0	±SEM	Differences: P value
Waist cm	-2.1	0.6	-6.1	1.2	**0.003***
Hip cm	-0.4	1.0	-4.4	1.4	**0.024***
Triceps mm	0.9	1.7	-6.9	3.4	**0.016***
Biceps mm	1.3	1.3	-10.4	3.3	**0.001***
Suprailiac mm	2.5	2.1	-12	2.3	**0.000***
Sub Scapular mm	2.2	1.8	-5.0	1.5	**0.008***
Skinfold sum mm	2.5	2.1	-12	2.3	**0.000***
Body Density	-0.004	0.001	0.011	0.002	**0.000***
Body fat %	1.9	0.9	-5.0	1.0	**0.000***
Plasma Apo A g / L	0.17	0.06	-0.04	0.04	**0.023***
Diet Intake:					
Monounsaturated. F % total fat	0.07	1.3	5.5	2.1	**0.034***
Saturated.F % total fat	0.9	1.1	-6.5	2.3	**0.004***
Mono:Sat:Ratio	-0.02	0.1	0.44	0.1	**0.002***

Table 4.60: Comparison of differences in diet intake, anthropometric and lipid measurement between the changes observed in Control diet and HMUFA diet male sub-groups at 3 months (compared to base-line). Only significant differences are shown.

4.9.2.3 Six months:

At six months time only diet intake was analysed. There were significant differences in diet intake between the two male sub-groups (Table 4.61).

Variable	Control diet 6 months	±SEM	HMUFA diet 6 months	±SEM	Differences: P value
BMR	1915	53	1665	78	**0.002***
monounsat.F % total fat	42.1	1.5	48.7	1.7	**0.006***
Saturated.F % total fat	36.4	1.3	29.8	1.6	**0.004***
mono:Sat:Ratio	1.18	0.1	1.68	0.1	**0.001***

Table 4.61: Comparison of the mean ± SEM of diet intake between HMUFA diet and Control diet in male sub-groups, at 6 months (only significant differences are shown).

There were significant changes in weight and diet intake when the differences between the changes observed at 6 months (compared to base-line) in the two male sub-groups were compared (Table 4.62).

Variable	Control diet 6-0	±SEM	HMUFA diet 6-0	±SEM	Differences: P value
Weight (Kg)	-3.0	2.3	-13.5	4.1	**0.006***
BMR	-50	48	-442	57	**0.000***
Monounsaturated. F% total fat	1.5	1.2	7.3	1.9	**0.016***
Mono:Sat:Ratio	0.17	0.1	0.62	0.1	**0.003***
Calcium, mg	74.2	74	-28	111	**0.012***

Table 4.62: Comparison of differences in weight and diet intake between the changes observed in Control diet and HMUFA diet male sub-groups at 6 months (compared to base-line). Only significant differences are shown.

4.9.2.4 Twelve months:

At twelve months time there were significant changes in lipid and lipoprotein, glucose uptake, and diet intake between the two sub-groups (Tables 4.63 and Figures 4.60 to 4.63).

Variable	Control diet 12 months	±SEM	HMUFA diet 12 months	±SEM	Differences: P value
NEFA mmol/L	0.90	0.1	0.38	0.1	**0.006***
HDL-C mmol/L	0.76	0.08	1.03	0.05	**0.028***
HDL-Apo A g/L	1.06	0.06	1.28	0.06	**0.038***
CETP%	53.2	1.2	26.5	1.4	**0.000***
VLDL-Apo B g/L	0.21	0.03	0.11	0.02	**0.046***
Glucose uptake $mg.m^{-2}.min^{-1}$	90	19	188	20	**0.005***
Monounsat.F % total fat	43	1.4	49.8		**0.009***
Poly:sat. ratio	0.52	0.1	0.81	0.1	**0.002***
Saturated.F % total fat	37.8	0.9	28.1	1.5	**0.000***
Mono:Sat ratio	1.14	0.04	1.83	0.2	**0.000***
Saturated.Fat.g	27.9	2.2	19	3.2	**0.028***
Vitamin C, mg	137	18	74	10	**0.020***

Table 4.63: Comparison of the mean ± SEM of diet intake, glucose uptake, lipid, and lipoprotein measurement between HMUFA diet and Control diet male sub-groups. Only significant values are shown.

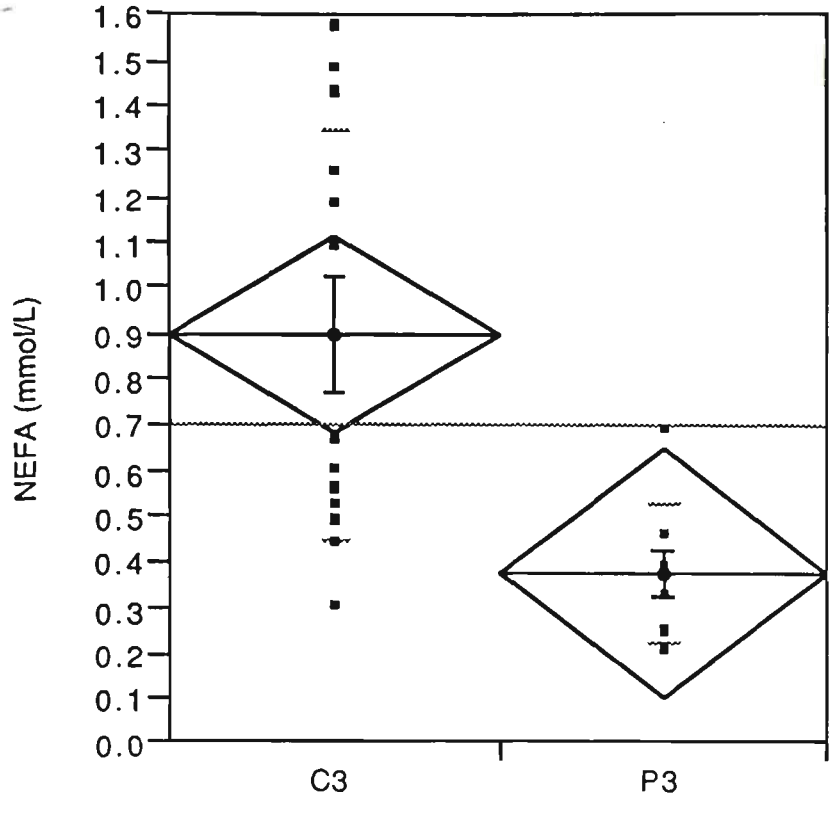

Figure 4.60: Comparison of the mean ± SEM of NEFA in Control (C3) and HMUFA (P3) male sub-group at 12 months (P=0.006).*

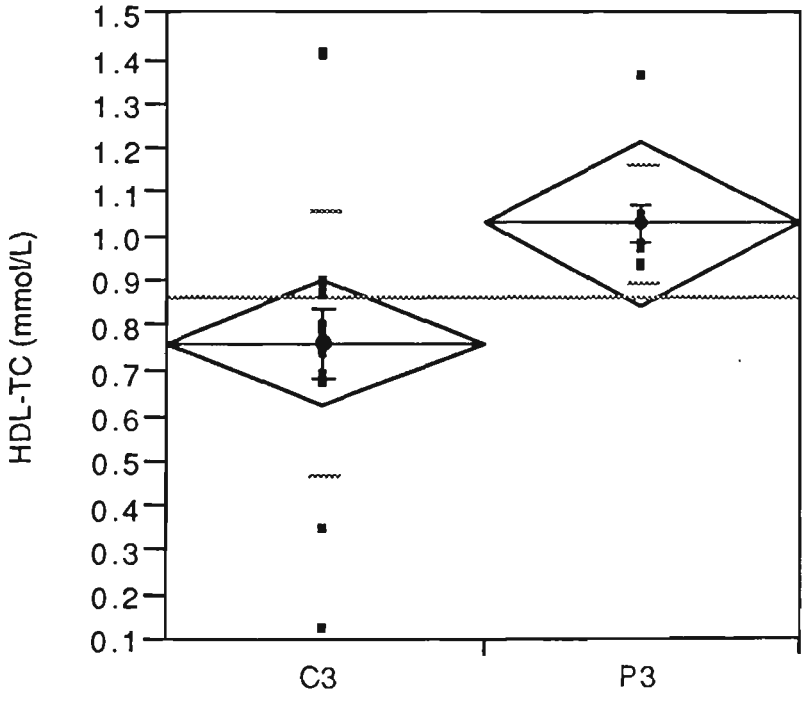

Figure 4.61: Comparison of the mean ± SEM of HDL-C in Control (C3) and HMUFA (P3) male sub-group at 12 months (P=0.028).*

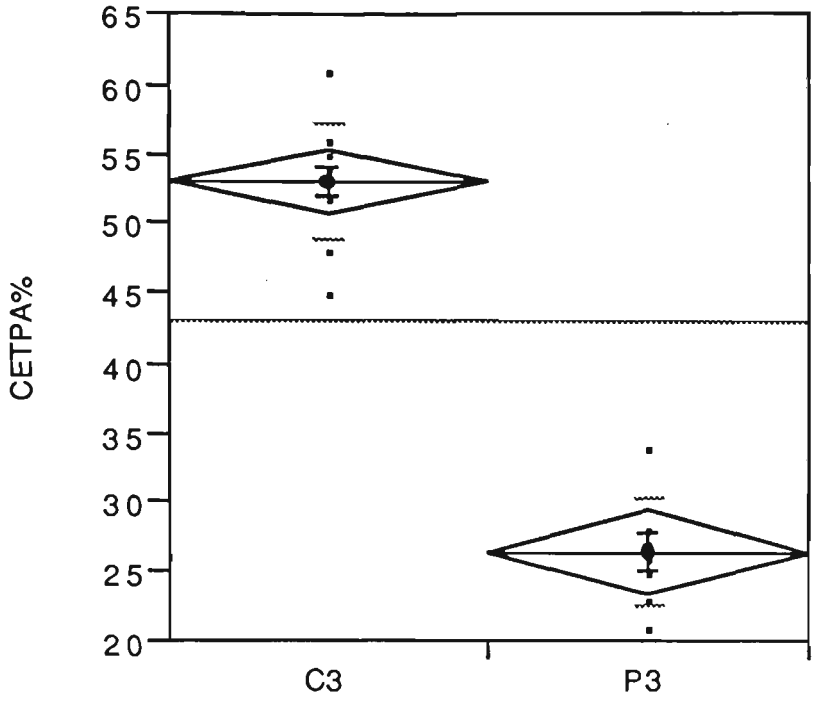

Figure 4.62: Comparison of the mean ± SEM of CETP activities (%) in Control (C3) and HMUFA (P3) male sub-group at 12 months (P=0.000).*

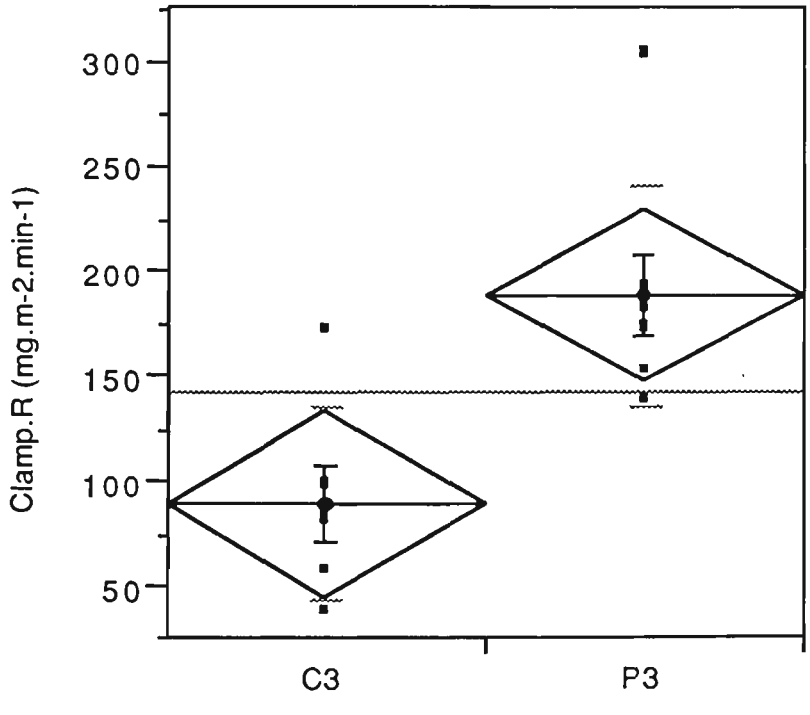

Figure 4.63: Comparison of the mean ± SEM of glucose uptake (Clamp R, $mg.m^{-2}.min^{-1}$) in Control (C3) and HMUFA (P3) male sub-group at 12 months (P=0.005).*

There were significant changes in the type of fat intake in the diet when the differences between the changes observed at 12 months (compared to base-line) in the two male sub-groups were compared (Table 4.64).

Variable	Control diet 12-0	±SEM	HMUFA diet 12-0	±SEM	Differences: P value
Monounsaturated.F % total fat	2.5	1.2	7.6	1.5	0.012*
Saturated.F % total fat	-2.3	1.2	-8.6	1.6	0.004*
Poly:sat. ratio	0.13	0.1	0.67	0.1	0.000*

Table 4.64: Comparison of differences in the fat intake in the diet between the changes observed in Control diet and HMUFA diet male sub-groups at 12 months (compared to base-line). Only significant differences are shown.

In comparison of changes within the group, there were significant changes in diet intake, anthropometric, lipid, and lipoprotein measurement in both male sub-groups (Tables 4.65 and 4.66). The Control diet male sub-group had significant decrease in saturated fat intake and increase in poly:sat ratio at 6 months time. No other significant changes were noted at 3 or 12 months in diet intake in Control group.

	Control (P value)		HMUFA (P value)	
Variable	3-0	12-0	3-0	12-0
Diastolic BP, mmHg	0.818	0.777	0.552	↓0.037*
Biceps, mm	0.567	0.417	↓0.021*	↓0.031*
Triceps, mm	0.742	0.561	0.079	↓0.031*
Suprailiac, mm	0.561	0.895	↓0.011*	↓0.035*
Skinfold sum mm	0.430	0.397	↓0.007*	0.014*
Density	0.284	0.136	↑0.025*	↑0.018*
Body fat %	0.274	0.130	↓0.040*	↓0.019*
IDL-Apo B g/L	0.420	0.496	↑0.032*	0.068
HDL-Apo A g/L	↑0.004*	0.666	0.105	↑0.045*
NEFA, mmol/L	0.156	↑0.000*	0.616	0.731
CETP%	0.106	↑0.000*	0.231	↓0.007*

*Table 4.65: The P value results of comparison within the male sub-groups at 3 months (3-0) and 12 months (12-0) in anthropometric and lipid measurement. The significant values are shown with *.*

	Control (P value)	HMUFA (P value)		
Variable	6-0	3-0	6-0	12-0
Estimate intake Kcal	0.785	↓0.046*	0.565	0.510
Monounsaturated F % total fat	0.611	↑0.045*	↑0.006*	↑0.007*
CHO, E%	0.947	0.982	0.728	↓0.038*
Simple CHO%	0.940	0.080	0.260	↑0.010*
Complex CHO%	0.940	0.080	0.260	↓0.010*
Dietary fibre, g	0.515	↓0.013*	0.798	0.693
Starch, g	0.871	↓0.007*	↓0.031*	↓0.014*
Saturated F, % total fat	↓0.014*	↓0.051*	↓0.010*	↓0.030*
g	0.555	↓0.048*	0.712	0.171
Mono:sat:ratio	0.058	↑0.017*	↑0.001*	↑0.019*
Poly:sat. ratio	↑0.039*	0.194	↑0.039*	↑0.012*
Phosphorus, mg	0.737	↓0.024*	0.741	0.790
RDI%	0.736	↓0.024*	0.743	0.789
Magnesium, mg	0.761	↓0.008*	0.766	0.620
Sodium, mg	0.417	↓0.023*	0.060	0.372
Iron, mg	0.378	↓0.004*	0.292	0.261
RDI%	0.476	↓0.004*	0.287	0.270

*Table 4.66: The P value results of comparison within the male sub-group at 3 months (3-0), 6 months (6-0) and 12 months (12-0) in daily energy and mineral intake. The significant values are shown with *.*

4.9.3 Female sub-group:

4.9.3.1 Base-line:

At base-line (0 time), there were no significant differences between Control diet and HMUFA diet, in anthropometric measurements, lipid and lipoproteins measurements, indices of glucose metabolism, past history, medication intake, indices of insulin sensitivity obtained by the euglycemic clamp technique. But there were significant differences in polyunsaturated fat intake (%), poly:sat. ratio, and Niacin intake in the diet (Table 4.67).

Variable	Control diet 0 time	±SEM	HMUFA diet 0 time	±SEM	Differences: P value
Poly.F % total fat	26	2.8	19	1.1	**0.017***
Poly:sat. ratio	0.72	0.1	0.50	0.03	**0.020***
Niacin, mg	25.4	2.9	35.2	2.4	**0.019***

Table 4.67: Comparison of the mean ± SEM of diet intake between HMUFA diet and Control diet in female sub-group, at 0 time (only significant differences are shown).

4.9.3.2 Three months:

At 3 months time also there were no significant differences between Control diet and HMUFA diet, in anthropometric measurements, lipid and lipoproteins measurements, indices of glucose metabolism, or medication intake. But there were significant changes in euglycemic clamp results, and diet intake between the two female sub-groups (Table 4.68 & Figure 4.64).

There were significant changes in hip circumflex, glucose uptake, fat intake in the diet (%), mono:sat ratio, and zinc intake when the differences between the changes observed at 3 months (compared to base-line) in the two female sub-groups were compared (Table 4.69).

Variable	Control diet 3 months	±SEM	HMUFA diet 3 months	±SEM	Differences: P value
Weight, Kg	72.9	4.6	88.1	4.8	**0.040***
BSA	1.7	0.04	1.9	0.06	**0.021***
BMR	1432	42	1588	49	**0.035***
Diet: Fat intake %	21.8	4.0	35	1.8	**0.013***
CHO% intake	57	4.8	46	1.7	**0.024***
monounsat.F % total fat	38.3	2.2	47	1.4	**0.002***
Mono:Sat:Ratio	1.04	0.1	1.52	0.1	**0.014***
Monounsat.F .g	11.7	3.1	22.9	3.6	**0.034***

Table 4.68: Comparison of the mean ± SEM of anthropometric measurement and diet intake between HMUFA diet and Control diet in female sub-group, at 3 months (only significant differences are shown).

Variable	Control diet 3-0	±SEM	HMUFA diet 3-0	±SEM	Differences: P value
Hip, cm	0.8	1.4	-2.5	1.0	**0.040***
Glucose uptake	-23	10	42	18	**0.014***
Diet intake:					
Fat intake %	1.1	1.3	-1.9	0.6	**0.030***
Mono:Sat:Ratio	0.08	0.1	0.52	0.1	**0.043***
Zinc, mg	0.14	0.8	-2.5	0.9	**0.041***

Table 4.69: Comparison of differences in the hip circumflex, glucose uptake, and diet intake between the changes observed in Control diet and HMUFA diet female sub-groups at 3 months (compared to base-line). Only significant differences are shown.

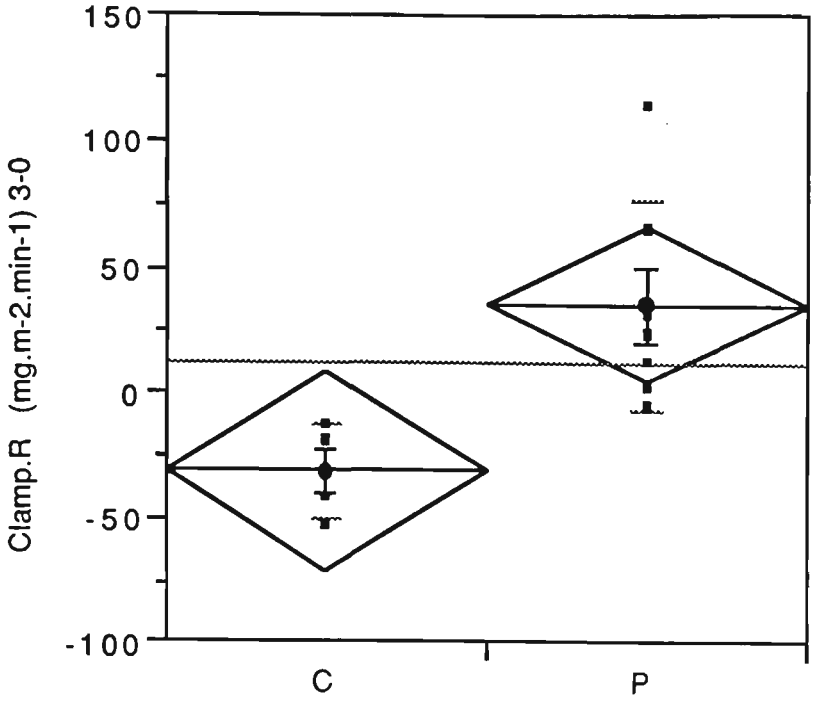

Figure 4.64: Comparison of differences in the glucose uptake between the changes observed in Control diet and HMUFA diet female sub-groups at 3 months (compared to base-line) (P=0.014).*

4.9.3.3 Six months:

At Six months time only diet intake were analysed. There were significant differences in diet intake between the two female sub-groups (Table 4.70).

Variable	Control diet 6 months	±SEM	HMUFA diet 6 months	±SEM	Differences: P value
Cholesterol, mg	135	36	218	18	**0.031***
Riboflavin, mg	27.7	5.1	41	3.5	**0.038***
mono:Sat:Ratio	1.07	0.1	1.7	0.1	**0.001***
Magnesium, mg	214	26	318	28	**0.024***
Fat, g	39.1	9.1	67.2	7.0	**0.024***
Polyunsat.F .g	7.2	1.7	13.3	1.6	**0.024***

Table 4.70: Comparison of the mean ± SEM of diet intake between HMUFA diet and Control diet in female sub-group, at 6 months (only significant differences are shown).

4.9.3.4 Twelve months:

At twelve months time there were significant changes in anthropometric measurements, lipid and lipoprotein measurements, and diet intake between the two female sub-groups (Tables 4.71, 4.72 and Figures: 4.65 to 4.68).

Variable	Control diet 12 months	±SEM	HMUFA diet 12 months	±SEM	Differences: P value
Weight, Kg	72.6	4.4	91.8	3.4	**0.002***
Diastolic B.P mmHg	82.2	3.2	72.2	2.2	**0.015***
Mean Arterial Pressure	109	4.0	99	3.0	**0.048***
Waist, cm	93.2	4.5	104.7	2.9	**0.036***
Hip, cm	107.9	3.6	120	2.3	**0.008***
BMI	29.8	1.9	35	1.0	**0.017***
BSA	1.72	0.05	1.96	0.05	**0.002***
BMR	1423	39	1631	41	**0.002***
Biceps, mm	18.4	1.4	24.8	1.9	**0.027***
NEFA mmol/L	0.7	0.1	0.4	0.04	**0.002***
IDL-C mmol/L	0.12	0.02	0.06	0.01	**0.019***
IDL-FC mmol/L	0.05	0.01	0.01	0.003	**0.010***
HDL-C, mmol/L	0.77	0.1	1.1	0.1	**0.011***
HDL Apo A g/L	1.17	0.1	1.43	0.1	**0.021***
CETP%	53.7	3.6	34.1	3.6	**0.001***

Table 4.71: Comparison of the mean ± SEM of anthropometric, lipid and lipoprotein measurements between HMUFA diet and Control diet in female sub-group, at 12 months (only significant differences are shown).

Variable	Control diet 12 months	±SEM	HMUFA diet 12 months	±SEM	Differences: P value
Monounsat.F % total fat	40.7	1.3	49.5	1.1	**0.000***
Saturated.F % total fat	38.7	1.5	30.3	1.6	**0.002***
Mono:Sat:Ratio	1.07	0.1	1.7	0.1	**0.001***
Protein.g	66.8	7.9	84.8	3.1	**0.030***
Thiamine, mg	0.99	0.1	1.42	0.1	**0.022***
Niacin, mg	27.1	3.0	34.1	1.1	**0.025***
Sodium, mg	1782	253	2538	207	**0.031***
Monounsat.F g	16.6	3.2	27.9	3.2	**0.024***

Table 4.72: Comparison of the mean ± SEM of diet intake between HMUFA diet and Control diet in female sub-group, at 12 months (only significant differences are shown).

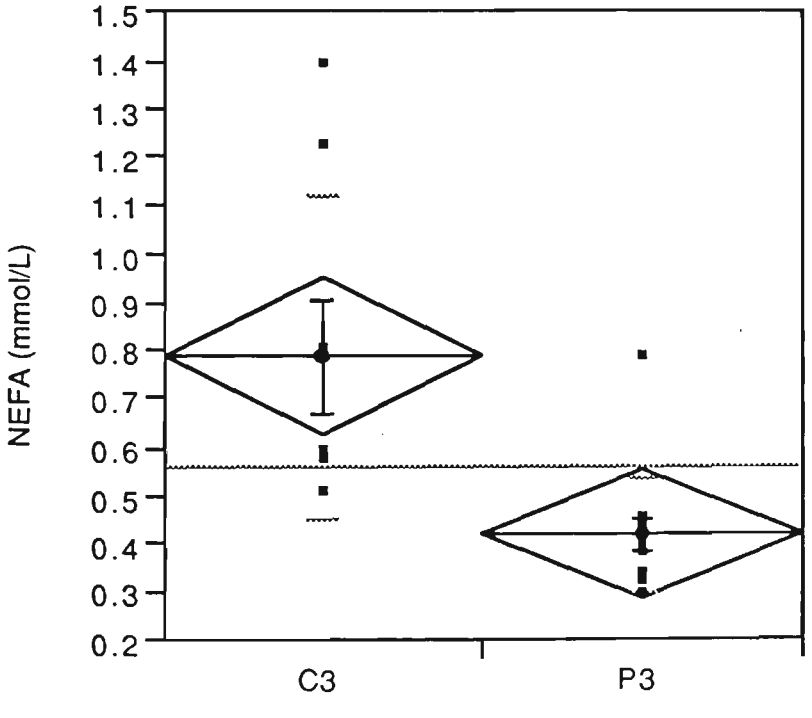

Figure 4.65: Comparison of the mean ± SEM of NEFA in Control (C3) and HMUFA (P3) female sub-group at 12 months (P=0.002).*

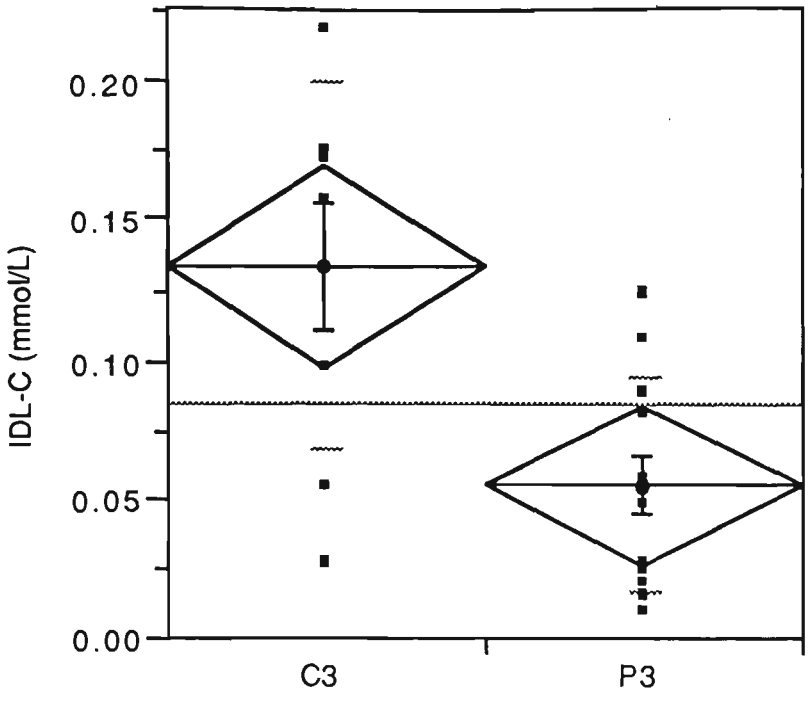

Figure 4.66: Comparison of the mean ± SEM of IDL-C in Control (C3) and HMUFA (P3) female sub-group at 12 months (P=0.019).*

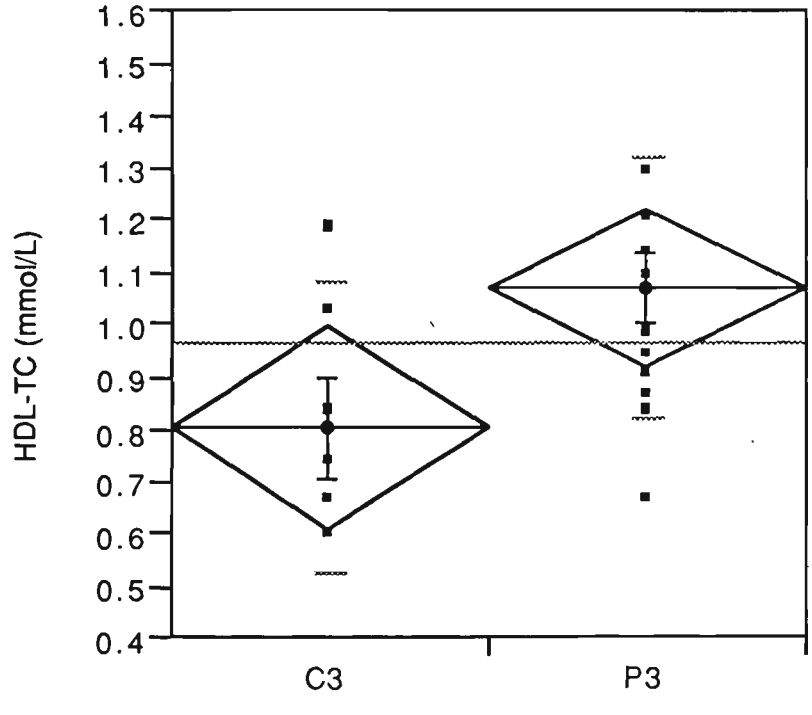

Figure 4.67: Comparison of the mean ± SEM of HDL-C in Control (C3) and HMUFA (P3) female sub-group at 12 months (P=0.011).*

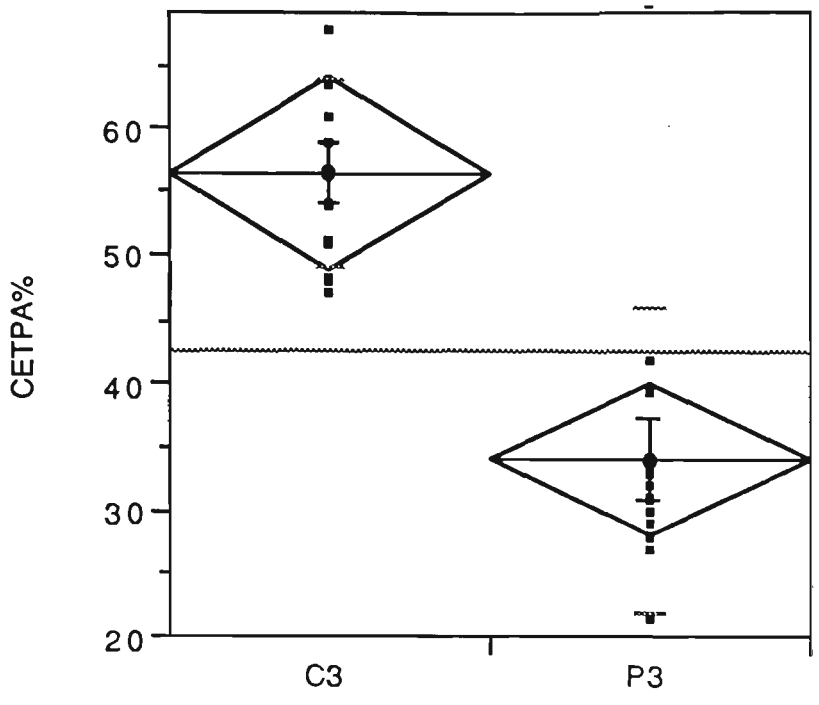

Figure 4.68: Comparison of the mean ± SEM of CETP activities (%) in Control (C3) and HMUFA (P3) female sub-group at 12 months (P=0.001).*

There were significant changes in NEFA, HDL-C, CETPA%, glucose uptake, diet intake when the differences between the changes observed at 12 months (compared to base-line) in the two female sub-groups were compared (Table 4.73).

Variable	Control diet 12-0	±SEM	HMUFA diet 12-0	±SEM	Differences: P value
NEFA , mmol/L	0.27	0.1	-0.13	0.04	**0.004***
HDL-C, g/L	-0.33	0.1	0.04	0.1	**0.013***
CETP%	17.3	5.0	-4.0	3.5	**0.002***
Glucose uptake $mg.m^{-2}.min^{-1}$	-56	21	50	20	**0.006***
Diet:					
Polyunsaturated.F%	-6.5	2.3	0.6	1.3	**0.016***
Saturated.F%	0.5	2.8	-9.3	1.8	**0.006***
Mono:Sat:Ratio	0.1	0.1	0.7	0.1	**0.012***
Niacin, mg	3.4	1.6	-3.5	1.9	**0.018***
Zinc, mg	0.14	0.8	-2.7	0.6	**0.034***

Table 4.73: Comparison of differences in NEFA, HDL-C, CETPA%, glucose uptake, diet intake between the changes observed in Control diet and HMUFA diet female sub-groups at 3 months (compared to base-line). Only significant differences are shown.

In comparison of changes within the group at 12 months, there were significant changes in blood pressure, lipid and lipoprotein measurements, and diet intake (Table 4.74, 4.75). No significant changes were noted within the group at 3 months or 6 months (data not shown).

	Control (P value)	HMUFA (P value)
Variable	12-0	12-0
Diastolic B.P	0.405	↓0.010*
MAP , mmHg	0.561	↓0.043*
IDL-C, mmol/L	0.977	↓0.006*
IDL-TG, mmol/L	0.644	↓0.022*
IDL-FC, mmol/L	0.950	↓0.014*
VLDL-TGmmol/l	↑0.0402*	0.281
HDL-C, mmol/L	↓0.016*	0.544
NEFA, mmol/l	↑0.033*	↓0.037*

*Table 4.74: The P value results of comparison within the group at 12 months (12-0) in blood pressure, lipid and lipoprotein measurements. The significant values are shown with *.*

	Control (P value)	HMUFA (P value)		
Variable	12-0	3-0	6-0	12-0
Monounsaturated F E%	↑0.007*	↑0.000*	↑0.002*	↑0.028*
Saturated F., E%	0.992	↓0.000*	↓0.003*	↓0.004*
g	0.548	↓0.032*	↓0.026*	↓0.051
Simple CHO%	0.692	0.871	0.337	↓0.003*
Complex CHO%	0.692	0.871	0.337	↑0.003*
Polyunsaturated F. E%	↓0.030*	0.490	0.370	0.551
Mono:sat:ratio	0.283	↑0.000*	↑0.002*	↑0.005*
Poly:sat. ratio	0.211	↑0.009*	↑0.023*	↑0.017*
Thiamine, mg	0.589	0.934	↑0.049*	0.327
Vitamin C, mg	0.688	0.223	0.611	↓0.041*

*Table 4.75: The P value results of comparison within the group at 3 months (3-0), 6 months (6-0) and 12 months (12-0) in daily energy and vitamin intake. The significant values are shown with *.*

4.10 Correlations:

Correlations measured in this study include:

a- Pairwise correlation during 3 (3-0) months in both groups ;

b- Pairwise correlation during 12 (12-0) months in both groups ;

Clamp study sub-group:

c- Pairwise correlation during 3 (3-0) months in clamp study sub-group ;

d- Pairwise correlation during 12 (12-0) months in clamp study sub-group .

This section shows the correlation between variables and changes in variables during 12 months. Only some of the significant correlations are shown here ($P < 0.05$). Important correlations are discussed elsewhere in the thesis.

Some of the pairwise correlations, with significant probability of less than 0.05 during 12 months, in both groups and in clamp study sub-group are as follows:

4.10.1 Pairwise correlations in both groups:

CORRELATIONS 12-0

Variable	by Variable	Correlation	Significant Probability
D.B.P-12-0	weight-12-0	0.3025	0.0487
waist-12-0	S.B.P-12-0	0.3934	0.0091
waist 12-0	D.B.P-12-0	0.3343	0.0285
waist -12-0	M.A.P-12-0	0.3899	0.0098
hip-12-0	S.B.P-12-0	0.4147	0.0057
hip -12-0	M.A.P-12-0	0.3791	0.0122
BMI-12-0	D.B.P-12-0	0.3105	0.0427
B.S.A-12-0	D.B.P-12-0	0.3132	0.0408
B.S.A-12-0	M.A.P-12-0	0.3102	0.0429
biceps-12-0	D.B.P-12-0	0.3840	0.0110
biceps -12-0	M.A.P-12-0	0.3343	0.0285
biceps -12-0	waist-12-0	0.3400	0.0257
biceps -12-0	hip-12-0	0.4669	0.0016
biceps -12-0	BMI-12-0	0.3217	0.0354
biceps 12-0	triceps-12-0	0.5812	0.0000
sub scapular-12-0	D.B.P-12-0	0.3168	0.0384
sub scapular -12-0	M.A.P-12-0	0.3177	0.0379
sub scapular -12-0	triceps-12-0	0.5172	0.0004
sub scapular -12-0	biceps-12-0	0.3431	0.0243
suprailiac-12-0	weight-12-0	0.3362	0.0275
suprailiac -12-0	D.B.P-12-0	0.3323	0.0295
suprailiac -12-0	hip-12-0	0.3156	0.0392
suprailiac -12-0	BMI-12-0	0.3144	0.0400
skinfold sum-12-0	weight-12-0	0.3160	0.0390
skinfold sum -12-0	D.B.P-12-0	0.4156	0.0056
skinfold sum -12-0	M.A.P-12-0	0.3312	0.0300
skinfold sum -12-0	hip-12-0	0.3854	0.0107
skinfold sum -12-0	BMI-12-0	0.3081	0.0444
c-peptide-12-0	weight-12-0	0.3736	0.0136

c-peptide -12-0	waist-12-0	0.3869	0.0104
c-peptide -12-0	hip-12-0	0.4270	0.0043
c-peptide -12-0	BMI-12-0	0.3906	0.0096
c-peptide -12-0	BMR-12-0	0.3468	0.0227
c-peptide -12-0	suprailiac-12-0	0.3126	0.0412
c-peptide -12-0	insulin-12-0	0.9032	0.0000
FBG-12-0	hip-12-0	0.4120	0.0060
FBG-12-0	B.S.A-12-0	0.3139	0.0404
plasma-C-12-0	subscapular-12-0	0.3018	0.0492
plasma-TG-12-0	waist-12-0	0.4022	0.0075
plasma-TG-12-0	BMI-12-0	0.3511	0.0210
plasma-TG-12-0	subscapular -12-0	0.3497	0.0215
plasma-TG-12-0	HB A1c%-12-0	0.3567	0.0189
plasma-TG-12-0	c-peptide-12-0	0.3038	0.0476
plasma-TG-12-0	FBG-12-0	0.3378	0.0267
plasma apo A-12-0	B.S.A-12-0	-0.3249	0.0335
plasma apo B-12-0	B.S.A-12-0	-0.4881	0.0009
NEFA-12-0	S.B.P-12-0	0.3809	0.0117
NEFA-12-0	D.B.P-12-0	0.4714	0.0014
NEFA-12-0	M.A.P-12-0	0.4506	0.0024
NEFA-12-0	triceps-12-0	0.4506	0.0024
NEFA-12-0	biceps-12-0	0.2120	0.1724
NEFA-12-0	sub scapular-12-0	0.4670	0.0016
NEFA-12-0	suprailiac-12-0	0.2071	0.1826
NEFA-12-0	skinfold sum-12-0	0.4227	0.0047
NEFA-12-0	body density-12-0	-0.2558	0.0978
NEFA-12-0	body fat %-12-0	0.2594	0.0929
NEFA-12-0	Hb A1c%-12-0	0.4284	0.0042
NEFA-12-0	plasma-TG-12-0	0.5163	0.0004
VLDL-C-12-0	NEFA -12-0	0.4304	0.0040
VLDL-TG-12-0	S.B.P-12-0	0.3212	0.0357
VLDL-TG-12-0	D.B.P-12-0	0.3076	0.0448
VLDL-TG-12-0	M.A.P-12-0	0.3355	0.0279
VLDL-TG-12-0	NEFA-12-0	0.5908	0.0000
VLDL-FC-12- 0	waist-12-0	0.3178	0.0378
VLDL-FC-12- 0	BMR-12-0	0.3689	0.0149
VLDL-FC-12- 0	NEFA-12-0	0.4786	0.0012
IDL-C-12-0	Body Density-12-0	-0.3021	0.0490
IDL-C-12-0	body fat %-12-0	0.3055	0.0463
IDL-FC12-0	waist-12-0	0.3137	0.0405
IDL-FC12-0	suprailiac-12-0	0.3022	0.0489
HDL-apo A-12-0	hip-12-0	-0.3287	0.0314
HDL-apo A-12-0	triceps-12-0	-0.3269	0.0324

HDL-apo A-12-0	biceps-12-0	-0.3959	0.0086
HDL-apo A-12-0	suprailiac-12-0	-0.3402	0.0256
HDL-apo A-12-0	skinfold sum-12-0	-0.3613	0.0173
HDL-apo A-12-0	body density-12-0	0.4248	0.0045
HDL-apo A-12-0	body fat %-12-0	-0.4289	0.0041
CETPA%-12-0	D.B.P-12-0	0.4239	0.0051
CETPA%-12-0	M.A.P-12-0	0.3812	0.0127
CETPA%-12-0	triceps-12-0	0.4304	0.0044
CETPA%-12-0	sub scapular-12-0	0.4535	0.0026
CETPA%-12-0	suprailiac-12-0	0.4667	0.0018
CETPA%-12-0	skinfold sum-12-0	0.5399	0.0002
CETPA%-12-0	body density-12-0	-0.5533	0.0001
CETPA%-12-0	body fat %-12-0	0.5536	0.0001
CETPA%-12-0	FBG-12-0	0.3673	0.0167
CETPA%-12-0	plasma-TG-12-0	0.3604	0.0191
CETPA%-12-0	NEFA-12-0	0.4636	0.0020
CETPA%-12-0	VLDL-C-12-0	0.3311	0.0322
CETPA%-12-0	VLDL-TG-12-0	0.3418	0.0267
CETPA%-12-0	VLDL-APO B-12-0	0.3411	0.0271
CETPA%-12-0	VLDL-FC-12- 0	0.3128	0.0437
CETPA%-12-0	IDL-C-12-0	0.3724	0.0151
CETPA%-12-0	HDL-TC-12-0	-0.3951	0.0096
CETPA%-12-0	HDL-apo A-12-0	-0.4131	0.0065
glucose uptake-12-0	S.B.P-12-0 -	0.4123	0.0506
glucose uptake-12-0	hip-12-0	-0.4082	0.0532
glucose uptake-12-0	sub scapular-12-0	-0.4552	0.0291
glucose uptake-12-0	FBG-12-0	-0.4932	0.0168
glucose uptake-12-0	plasma-C-12-0	-0.5099	0.0129
glucose uptake-12-0	plasma apo B-12-0	-0.4811	0.0201
glucose uptake-12-0	CETPA%-12-0	-0.5923	0.0037
fat intake % 12-0	S.B.P-12-0	-0.4962	0.0008
fat intake % 12-0	M.A.P-12-0	-0.4184	0.0058
fat intake % 12-0	waist-12-0	-0.3983	0.0090
fat intake % 12-0	hip-12-0	-0.3398	0.027
fat intake % 12-0	B.S.A-12-0	-0.3421	0.0266
fat intake % 12-0	Hb A1c%-12-0	-0.3249	0.0358
fat intake % 12-0	FBG-12-0	-0.4460	0.0031
fat intake % 12-0	NEFA-12-0	-0.2962	0.0569
fat intake % 12-0	VLDL-TG-12-0	-0.3321	0.0316
CHO%12-0	VLDL-C-12-0	-0.3749	0.0144
CHO%12-0	VLDL-APO B-12-0	-0.3049	0.0496
CHO%12-0	IDL-C-12-0	-0.3262	0.0350
CHO%12-0	fat intake %12-0	-0.5551	0.0001
poly% 12-0	body density-12-0	0.3430	0.0262
poly% 12-0	body fat %-12-0	-0.3455	0.0250
poly% 12-0	IDL-C-12-0	-0.5401	0.0002
poly% 12-0	IDL-TG12-0	-0.3142	0.0427
poly% 12-0	HDL-TC-12-0	0.3452	0.0252

poly% 12-0	HDL-apo A-12-0	0.3616	0.0186
poly% 12-0	glucose uptake12-0	0.4151	0.0489
mono% 12-0	S.B.P-12-0	-0.4218	0.0054
mono% 12-0	D.B.P-12-0	-0.3928	0.0101
mono% 12-0	M.A.P-12-0	-0.4351	0.0040
mono% 12-0	suprailiac-12-0	-0.3120	0.0443
mono% 12-0	skinfold sum-12-0	-0.3154	0.0419
mono% 12-0	NEFA-12-0	-0.4129	0.0066
sat.fat intake %12-0	D.B.P-12-0	0.3079	0.0473
sat.fat intake %12-0	triceps-12-0	0.3745	0.0146
sat.fat intake %12-0	suprailiac-12-0	0.3894	0.0108
sat.fat intake %12-0	skinfold sum-12-0	0.3967	0.0093
sat.fat intake %12-0	body density-12-0	-0.4216	0.0054
sat.fat intake %12-0	body fat %-12-0	0.4229	0.0053
sat.fat intake %12-0	NEFA-12-0	0.4020	0.0083
sat.fat intake %12-0	IDL-C-12-0	0.4818	0.0012
sat.fat intake %12-0	IDL-TG12-0	0.5022	0.0007
sat.fat intake %12-0	CETPA%-12-0	0.3953	0.0105
sat.fat intake %12-0	poly% 12-0	-0.6310	0.0000
sat.fat intake %12-0	mono% 12-0	-0.6187	0.0000
poly:sat. ratio-12-0	triceps-12-0	-0.3162	0.0413
poly:sat. ratio-12-0	suprailiac-12-0	-0.3154	0.0419
poly:sat. ratio-12-0	skinfold sum-12-0	-0.3214	0.0380
poly:sat. ratio-12-0	body density-12-0	0.4428	0.0033
poly:sat. ratio-12-0	body fat %-12-0	-0.4443	0.0032
poly:sat. ratio-12-0	IDL-C-12-0	-0.5306	0.0003
poly:sat. ratio-12-0	IDL-TG12-0	-0.3934	0.0100
poly:sat. ratio-12-0	HDL-apo A-12-0	0.3173	0.0406
poly:sat. ratio-12-0	CETPA%-12-0	-0.4116	0.0075
poly:sat. ratio-12-0	glucose uptake-12-00.5094		0.0130
mono:sat. ratio12-0	M.A.P-12-0	-0.3163	0.0413
mono:sat. ratio12-0	triceps-12-0	-0.3445	0.0255
mono:sat. ratio12-0	suprailiac-12-0	-0.4553	0.0024
mono:sat. ratio12-0	skinfold sum-12-0	-0.4165	0.0061
mono:sat. ratio12-0	body density-12-0	0.4264	0.0049
mono:sat. ratio12-0	body fat %-12-0	-0.4238	0.0052
mono:sat. ratio12-0	plasma apo A-12-0	-0.3045	0.0499
mono:sat. ratio12-0	NEFA-12-0	-0.4518	0.0027
mono:sat. ratio12-0	IDL-TG12-0	-0.3653	0.0174
mono:sat. ratio12-0	CETPA%-12-0	-0.4550	0.0028
mono:sat. ratio12-0	glucose uptake-12-0	0.4585	0.0278
mono:sat. ratio12-0	poly:sat. ratio-12-0	0.6475	0.0000
cholestrol,mg 12-0	FBG-12-0	-0.3667	0.0169
cholestrol,mg 12-0	plasma apo A-12-0	0.3077	0.0474
cholestrol,mg 12-0	plasma apo B-12-0	0.5317	0.0003
cholestrol,mg 12-0	VLDL-C-12-0	0.3645	0.0176
cholestrol,mg 12-0	VLDL-apo B-12-0	0.3619	0.0185
cholestrol,mg 12-0	fat intake %12-0	0.3552	0.0210
cholestrol,mg 12-0	CHO%12-0	-0.5605	0.0001

fat. g12-0	S.B.P-12-0	-0.3214	0.0379
fat.g 12-0	M.A.P-12-0	-0.3221	0.037
fat.g 12-0	B.S.A-12-0	-0.3733	0.0149
fat.g 12-0	NEFA-12-0	-0.4919	0.0009
sat.fat.g-12-0	Hb A1c%-12-0	-0.3411	0.0271
sat.fat.g-12-0	IDL-C-12-0	0.3723	0.0152
Mono.fat intake, g-12-0	S.B.P-12-0	-0.4456	0.0031
Mono.fat intake, g-12-0	D.B.P-12-0	-0.4037	0.0080
Mono.fat intake, g-12-0	M.A.P-12-0	-0.4541	0.0025
Mono.fat intake, g-12-0	B.S.A-12-0	-0.4093	0.0071
Mono.fat intake, g-12-0	FBG-12-0	-0.3180	0.0401
Mono.fat intake, g-12-0	NEFA-12-0	-0.4863	0.0011
Mono.fat intake, g-12-0	VLDL-TG-12-0	-0.3277	0.0341
Poly.fat intake, g12-0	NEFA-12-0	-0.3102	0.0456
canola%-12-0	SBP-12-0	-0.3224	0.0484
canola%-12-0	Hb A1c-12-0	-0.3982	0.0133
canola%-12-0	NEFA-12-0	-0.3330	0.0410
canola-g-12-0	smoking/day-12-0	0.4566	0.0045
canola-g-12-0	alcohol/w-12-0	0.3714	0.0236
canola-g-12-0	sub scapular-12-0	-0.3489	0.0343
canola-g-12-0	Hb A1c-12-0	-0.4267	0.0084
canola-g-12-0	NEFA-12-0	-0.3858	0.0183
canola-g-12-0	IDL-C-12-0	-0.3831	0.0193
canola-g-12-0	canola%-12-0	0.8795	0.0000
canola n-3 g,12-0	smoking/day-12-0	0.4566	0.0045
canola n-3 g,12-0	alcohol/w-12-0	0.3714	0.0236
canola n-3 g,12-0	sub scapular-12-0	-0.3489	0.0343
canola n-3 g,12-0	Hb A1c-12-0	-0.4267	0.0084
canola n-3 g,12-0	NEFA-12-0	-0.3858	0.0183
canola n-3 g,12-0	IDL-C-12-0	-0.3831	0.0193
canola n-3 g,12-0	IDL-TG12-0	-0.2962	0.0751
canola n-3 g,12-0	canola%-12-0	0.8795	0.0000
canola n-3 g,12-0	canola-g-12-0	1.0000	0.0000
n-6:n-3 ratio-12-0	smoking/day-12-0	-0.9195	0.0002
n-6:n-3 ratio-12-0	alcohol/w-12-0	-0.7294	0.0167
n-6:n-3 ratio-12-0	BMI-12-0	0.6586	0.0384
n-6:n-3 ratio-12-0	VLDL-TG-12-0	0.8247	0.0033
n-6:n-3 ratio-12-0	canola%-12-0	-0.9148	0.0002
n-6:n-3 ratio-12-0	canola-g-12-0	-0.9382	0.0001
n-6:n-3 ratio-12-0	canola n-3 g,12-0	-0.9382	0.0001

4.10.2 Pairwise correlations in Clamp study sub-group group during 12 months:

Variable	by Variable	Correlation	Significant Probability
waist-12-0	S.B.P-12-0	0.4968	0.0135
waist -12-0	D.B.P-12-0	0.5564	0.0047
waist -12-0	M.B.P-12-0	0.5590	0.0045
HIP-12-0	S.B.P-12-0	0.5922	0.0023
HIP-12-0	D.B.P-12-0	0.4265	0.0377
HIP-12-0	M.B.P-12-0	0.5516	0.0052
Subscapular-12-0	S.B.P-12-0	0.4094	0.0470
Subscapular-12-0	M.B.P-12-0	0.4136	0.0445
Hb A1c%-12-0	triceps-12-0	0.4054	0.0493
Hb A1c%-12-0	subscapular-12-0	0.4900	0.0151
insulin-12-0	hip-12-0	0.4346	0.0338
c-peptide-12-0	hip-12-0	0.4283	0.0368
FBG-12-0	subscapular-12-0	0.5516	0.0052
FBG-12-0	skinfold sum-12-0	0.4880	0.0155
FBG-12-0	density -12-0	-0.4183	0.0419
FBG-12-0	body fat%-12-0	0.4230	0.0394
plasma-C-12-0	subscapular-12-0	0.4591	0.0240
plasma-C-12-0	c-peptide-12-0	0.4603	0.0236
plasma-TG-12-0	waist-12-0	0.4066	0.0487
NEFA-12-0	S.B.P-12-0	0.4410	0.0310
NEFA-12-0	D.B.P-12-0	0.5155	0.0099
NEFA-12-0	M.B.P-12-0	0.5067	0.0115
NEFA-12-0	triceps-12-0	0.4829	0.0168
NEFA-12-0	Subscapular-12-0	0.6782	0.0003
NEFA-12-0	Skinfold Sum-12-0	0.5336	0.0072
NEFA-12-0	Hb A1c%-12-0	0.6443	0.0007
NEFA-12-0	FBG-12-0	0.6737	0.0003
NEFA-12-0	Plasma -TG-12-0	0.5692	0.0037
NEFA-12-0	Plasma Apo A-12-0	0.5020	0.0124
VLDL-TG-12-0	S.B.P-12-0	0.4560	0.0251
VLDL-TG-12-0	D.B.P-12-0	0.5626	0.0042
VLDL-TG-12-0	M.B.P-12-0	0.5382	0.0067

VLDL-TG-12-0	hip-12-0	0.4720	0.0199
VLDL-TG-12-0	NEFA-12-0	0.5359	0.0070
VLDL-FC-12- 0	D.B.P-12-0	0.4990	0.0131
VLDL-FC-12- 0	M.B.P-12-0	0.4695	0.0206
VLDL-FC-12- 0	waist-12-0	0.4303	0.0358
IDL-TG12-0	biceps-12-0	0.4442	0.0297
IDL-Apo B12-0	biceps-12-0	0.4769	0.0185
IDL-FC12-0	W:H R-12-0	0.5202	0.0092
HDL-APO A-12-0	hip-12-0	-0.4525	0.0264
CETPA%-12-0	S.B.P-12-0	0.4631	0.0261
CETPA%-12-0	M.B.P-12-0	0.4165	0.0481
CETPA%-12-0	subscapular-12-0	0.4668	0.0247
CETPA%-12-0	density -12-0	-0.4637	0.0259
CETPA%-12-0	body fat%-12-0	0.4586	0.0278
CETPA%-12-0	FBG-12-0	0.4675	0.0245
CETPA%-12-0	Plasma -TG-12-0	0.4510	0.0308
CETPA%-12-0	Plasma APO A-12-0	0.5466	0.0070
CETPA%-12-0	Plasma APO B-12-0	0.4688	0.0240
CETPA%-12-0	NEFA-12-0	0.5778	0.0039
CETPA%-12-0	VLDL-TG-12-0	0.4927	0.0169
CETPA%-12-0	IDL-C-12-0	0.4160	0.0484
glucose uptake-12-0	subscapular-12-0	-0.4552	0.0291
glucose uptake-12-0	FBG-12-0	-0.4932	0.0168
glucose uptake-12-0	plasma-C-12-0	-0.5099	0.0129
glucose uptake-12-0	plasma apo B-12-0	-0.4811	0.0201
glucose uptake-12-0	HDL-apo A-12-0	0.2140	0.3268
glucose uptake-12-0	CETPA%-12-0	-0.5923	0.0037
protein-12-0	D.B.P-12-0	0.5029	0.0122
protein -12-0	M.B.P-12-0	0.4687	0.0209
protein -12-0	biceps-12-0	0.4635	0.0226
protein -12-0	subscapular-12-0	0.4070	0.0484
protein -12-0	Skinfold Sum-12-0	0.4322	0.0349
protein -12-0	c-peptide-12-0	0.5754	0.0033
protein -12-0	NEFA-12-0	0.4282	0.0369
protein 12-0	VLDL-TG-12-0	0.4654	0.0219
protein -12-0	IDL-C-12-0	0.5123	0.0105
protein -12-0	IDL-TG12-0	0.5249	0.0084
fat intake %12-0	subscapular-12-0	-0.4580	0.0244
fat intake %12-0	FBG-12-0	-0.4096	0.0468
CHO%12-0	fat intake %12-0	-0.7787	0.0000
simple CHO12-0	c-peptide-12-0	0.4655	0.0219
simple CHO12-0	plasma-C-12-0	0.4641	0.0223
simple CHO12-0	plasma-TG-12-0	0.5114	0.0106
simple CHO12-0	plasma apo B-12-0	0.5641	0.0041

simple CHO12-0	VLDL-C-12-0	0.4985	0.0132
simple CHO12-0	VLDL-apo B-12-0	0.4281	0.0369
alcohol%12-0	plasma-TG-12-0	0.4295	0.0362
alcohol%12-0	plasma apo A-12-0	0.4320	0.0350
alcohol%12-0	VLDL-TG-12-0	0.4738	0.0193
alcohol%12-0	LDL-C12-0	-0.4584	0.0243
alcohol%12-0	HDL-TC-12-0	0.1942	0.3632
alcohol%12-0	CHO%12-0	-0.4267	0.0376
poly% 12-0	plasma apo B-12-0	-0.4960	0.0137
poly% 12-0	IDL-C-12-0	-0.6375	0.0008
poly% 12-0	glucose uptake-12-0	0.4151	0.0489
mono% 12-0	S.B.P-12-0	-0.4553	0.0254
mono% 12-0	D.B.P-12-0	-0.4866	0.0159
mono% 12-0	M.B.P-12-0	-0.5010	0.0126
mono% 12-0	alcohol-12-0	-0.4601	0.0237
mono% 12-0	triceps-12-0	-0.4165	0.0429
mono% 12-0	biceps-12-0	-0.4247	0.0386
mono% 12-0	skinfold sum-12-0	-0.4977	0.0133
mono% 12-0	density -12-0	0.5054	0.0118
mono% 12-0	body fat %-12-0	-0.5071	0.0114
mono% 12-0	plasma apo A-12-0	-0.2867	0.1743
mono% 12-0	IDL-C-12-0	-0.4650	0.0221
mono% 12-0	IDL-TG12-0	-0.5691	0.0037
mono% 12-0	CETPA%-12-0	-0.5035	0.0143
mono% 12-0	simple CHO12-0	0.4251	0.0384
mono% 12-0	complex CHO%12-0	-0.4251	0.0384
sat.fat intake %12-0	S.B.P-12-0	0.4553	0.0254
sat.fat intake %12-0	D.B.P-12-0	0.4252	0.0383
sat.fat intake %12-0	M.B.P-12-0	0.4712	0.0201
sat.fat intake %12-0	skinfold sum-12-0	0.4192	0.0415
sat.fat intake %12-0	density -12-0	-0.4077	0.0480
sat.fat intake %12-0	body fat %-12-0	0.4104	0.0464
sat.fat intake %12-0	plasma-C-12-0	0.4069	0.0485
sat.fat intake %12-0	plasma apo A-12-0	0.4583	0.0243
sat.fat intake %12-0	plasma apo B-12-0	0.4210	0.0405
sat.fat intake %12-0	NEFA-12-0	0.4049	0.0497
sat.fat intake %12-0	VLDL-TG-12-0	0.4858	0.0161
sat.fat intake %12-0	IDL-C-12-0	0.7603	0.0000
sat.fat intake %12-0	IDL-TG12-0	0.6528	0.0005
sat.fat intake %12-0	LDL-C12-0	0.4109	0.0461
sat.fat intake %12-0	CETPA%-12-0	0.5934	0.0028
sat.fat intake %12-0	protein-12-0	0.4958	0.0137
sat.fat intake %12-0	poly% 12-0	-0.7777	0.0000
sat.fat intake %12-0	mono% 12-0	-0.6952	0.0002
P:S:R-12-0	Plasma APO A-12-0	-0.5123	0.0105
P:S:R-12-0	Plasma APO B-12-0	-0.4745	0.0191
P:S:R-12-0	VLDL-TG-12-0	-0.4184	0.0419
P:S:R-12-0	IDL-C-12-0	-0.6726	0.0003
P:S:R-12-0	IDL-TG12-0	-0.4805	0.0175

P:S:R-12-0	LDL-C12-0	-0.4154	0.0435
P:S:R-12-0	CETPA%-12-0	-0.5869	0.0032
P:S:R-12-0	glucose uptake-12-0	0.5094	0.0130
mono:sat. ratio 12-0	alcohol-12-0	-0.4253	0.0383
mono:sat. ratio 12-0	skinfold sum-12-0	-0.4976	0.0134
mono:sat. ratio 12-0	density-12-0	0.5793	0.0030
mono:sat. ratio 12-0	body fat%-12-0	-0.5773	0.0031
mono:sat. ratio 12-0	Plasma APO A-12-0	-0.5250	0.0084
mono:sat. ratio 12-0	NEFA-12-0	-0.4132	0.0448
mono:sat. ratio 12-0	IDL-C-12-0	-0.6115	0.0015
mono:sat. ratio 12-0	IDL-TG12-0	-0.5672	0.0038
mono:sat. ratio 12-0	CETPA%-12-0	-0.6947	0.0002
mono:sat. ratio 12-0	glucose uptake-12-0	0.4585	0.0278
mono:sat. ratio 12-0	P:S:R-12-0	0.7627	0.0000
cholesterol mg12-0	Plasma Apo A-12-0	0.6403	0.0008
cholesterol mg12-0	VLDL-TG-12-0	0.4052	0.0495
cholesterol mg12-0	protein-12-0	0.4048	0.0498
cholesterol mg12-0	CHO%12-0	-0.4744	0.0192
cholesterol mg12-0	poly% 12-0	-0.4157	0.0433
cholesterol mg12-0	sat.fat intake %12-0	0.4174	0.0424
cholesterol mg12-0	P:S:R-12-0	-0.4386	0.0320
fat.mg12-0	hip-12-0	-0.4205	0.0407
fat.mg12-0	subscapular-12-0	-0.4160	0.0432
fat.mg12-0	c-peptide-12-0	-0.4173	0.0425
fat.mg12-0	NEFA-12-0	-0.4655	0.0219
CHO.g12-0	c-peptide-12-0	-0.4477	0.0283
sat.fat.g-12-0	alcohol/w-12-0	0.4220	0.0400
Mono.fat intake, g-12-0	weight-12-0	-0.4579	0.0244
Mono.fat intake, g-12-0	hip-12-0	-0.5021	0.0124
Mono.fat intake, g-12-0	BMR-12-0	-0.4833	0.0167
Mono.fat intake, g-12-0	Subscapular-12-0	-0.4448	0.0294
Mono.fat intake, g-12-0	c-peptide-12-0	-0.4220	0.0400
Mono.fat intake, g-12-0	NEFA-12-0	-0.5049	0.0119
Mono.fat intake, g-12-0	fat intake %12-0	0.6504	0.0006
Mono.fat intake, g-12-0	CHO%12-0	-0.4921	0.0146
Poly.fat intake, g12-0	NEFA-12-0	-0.4240	0.0389
Poly.fat intake, g12-0	VLDL-TG-12-0	-0.4482	0.0281
Poly.fat intake, g12-0	IDL-C-12-0	-0.4447	0.0294
Poly.fat intake, g12-0	sat.fat intake %12-0	-0.6135	0.0014
Poly.fat intake, g12-0	CHO.g12-0	0.4711	0.0202
Poly.fat intake, g12-0	Mono.fat intake, g-12-0	0.7975	0.0000

CHAPTER FIVE

DISCUSSION

Patients with NIDDM have abnormalities in the metabolism of both lipids and glucose which could contribute to diabetic complications. Although hypoglycaemic drugs may reduce plasma lipid and glucose levels, diet remains the cornerstone of therapy for NIDDM patients. The risk of developing NIDDM is associated with diet and it is thought it can be controlled to a large extent by food choices (Nettleton, 1995).

The desirable diet for patients with diabetes, however, has been a controversial subject for years (Garg et al., 1988). The approach to the dietary treatment of patients with diabetes mellitus has drastically changed in the last decade. Diabetologists are becoming increasingly more aware that the diabetic diet should not only help improve blood glucose control but also help prevent cardiovascular disease (Riccardi and Rivellese, 1991).

In this chapter we discuss the diet, dietary changes and associated changes in the experimental subjects.

In relation to CHO intake, there were no significant changes in either group during 3 or 12 months, and CHO intake was about 43 - 47% of total energy intake. This is close to the recommended CHO intake of 50% of total energy intake (ADA, 1994) for NIDDM patients. It seems clear though that the group, who had been given a high CHO low fat diet were not able to achieve the goal, certainly not able to maintain it for a year. No significant changes were noticed in the intake of simple

or complex CHO, total sugar, or starch during the study in either group. The changes in anthropometric measurements, blood pressure, insulin sensitivity, and lipid profiles in the HMUFA group can be therefore be ascribed to changes in the type of fat intake rather than CHO intake.

The Control diet group reduced their fat intake from 32% of total fat energy intake at 0 time to 31% at 12 months, and the HMUFA group increased their fat intake from 33% to 34% of fat energy intake respectively. Neither was a significant change and the total fat content in the two groups was similar. Total fat intake over 12 months was remarkably constant in both groups, in spite of attempted changes (liberalisation of fat intake in one group, a decrease in intake in the other). At 0 time, both group had 13% of their fat intake as saturated fat, falling slightly to 12% in the Control and 10% in the HMUFA group at 12 months.

Also at 0 time, both group had 7% of their fat intake as polyunsaturated fat. That fell slightly to 6% in the Control and remained the same in the HMUFA group at 12 months.

The major changes in fat intake involved monounsaturated fatty acids, saturated fatty acids, and n-3 and n-9 fatty acids from canola intake. During 12 months, the HMUFA group increased average intake of monounsaturated fatty acids (from 14% to 17% of total fat energy intake); canola n-3 (from 0.7 to 2.0 g); and canola n-9 (from 4.4 to 12.7 g) fatty acids; and decreased the intake of saturated fatty acids (from 39% to 29% of total fat energy intake). The ratio of monounsaturated to saturated fatty acid (Mono:Sat ratio) rose from 1.08:1 to 1.75:1 during the course of the study. The corresponding ratio for polyunsaturated fatty acids (Poly:Sat ratio) also rose from 0.53:1 to 0.74:1.

There were not any significant changes in polyunsaturated fatty acid or total n-6 fatty acid intake. These changes were apparent at 3 months and 6 months time and also in the sub-group which had euglycemic clamp studies. The canola n-3 fatty acid was calculated as canola intake (g) × 0.1 (canola oil has 10 g n-3, 20 g n-6, and 37 g n-9 fatty acids per 100 g) and total n-6 fatty acid was calculated as polyunsaturated intake (g) – n-3 (g) intake.

Over the 12 months of the study the Control group also increased their intake of monounsaturated fat (from 12% to 13% of total energy intake), reflected in modest but not significant changes in the Mono:Sat and the Poly:Sat ratios. There were not any significant changes in canola intake or total n-6 intake in the Control group.

In general, research has shown that a diet containing MUFA tends to be beneficial in subjects with NIDDM in relationship to lipid profiles and glycemic index (Garg et al., 1988; Mensink et al., 1989 Bonanome et al., 1991; Mata et al., 1992; Campbell et al., 1994., Lerman-Garber et al., 1994).

Long-chain n-3 fatty acids, found predominantly in fish oil, have a hypotriglyceridemic action (Friday et al., 1989; Hendra et al., 1990; Kasim, 1993). However fish oil has also been shown by some to have adverse effects on glycemic control in NIDDM (Glauber et al., 1988; Kasim et al., 1988; Friday et al., 1989; Borkman et al., 1989; Vessby 1989; Stacpole et al., 1989) and a tendency to elevate LDL-C (Jenkins et al., 1989; Kasim, 1989; Malasanos and Stacpoole, 1991; Connor et al., 1993). The n-3 fatty acid present in greatest quality in the diet of the HMUFA group was α-linolenic acid, a relatively short-chain (18:3) fatty acid which may (as a result of elongate and desaturate enzyme action) be metabolized to form long-chain n-3 fatty acids. Although n-3 intake significantly increased in the

HMUFA diet group, it is not clear what if any effect it had on metabolic parameters as changes could equally have been due to monounsaturated fatty acids (n-9).

Canola intake (g) was negatively correlated with subscapular skinfold measurement (r= -0.35, p=0.034), HbA1c (r= -0.43, p=0.008), NEFA (r= -0.39, p=0.018), and IDL-C (r= -0.38, p=0.019). Canola intake (%) was also negatively correlated with SBP (r= -0.32, p=0.048).

At 3 months there was a significant reduction of protein (g) intake in the diet of the HMUFA group, but this was not apparent at 12 months, when the protein intake was close to the recommended intake of 12-20% of total energy (ADA, 1993).

Waist and hip measurements:

Waist circumference was positively correlated with blood pressure (SBP: r=0.39, p=0.009; DBP: r=0.33, p=0.029; MAP: r=0.39, p=0.009). Hip circumference also was positively correlated with blood pressure (SBP: r=0.41, p=0.006; MAP: r=0.38, p=0.012).

Body fat distribution:

The body fat distribution may possibly be influenced by the type of fat in the diet (Hill et al., 1993). Although the HMUFA group in this study were fatter than the Control group at 0 time they were not significantly heavier. During 12 months their mean weight remained unchanged but the distribution of fat identified by skinfold measurements changed significantly in the HMUFA group. These changes were noted at 3 months and remained for the 12 months of the study. In the Control diet group there were no significant changes in fat distribution.

Skinfold measurement was also positively correlated with diastolic blood pressure (r=0.42, p=0.006) and mean arterial pressure (r=0.33, p=0.030). The body fat% was positively correlated with alcohol intake per week (r=0.35, p=0.023) and smoking per day (r=0.30, p=0.047).

Walker (1995) in a 3 months study did not report any significant changes in weight in NIDDM patients following a modified fat diet, compared to those following a Control diet. However he reported that the distribution of fat on the body influences the degree of insulin resistance that develops with obesity.

The distribution of body fat and the pattern of distribution of body fat appear more strongly related to NIDDM than simple measurements of body weight (Hartz et al., 1983; Shuman et al., 1986). Increased amounts of intra-abdominal adipose tissue are associated with various metabolic abnormalities associated with the insulin resistance syndrome (Banerji et al., 1995). There is also growing evidence that abdominal adiposity has a great role in the development of insulin resistance (Kohrt et al., 1993). In this study we did not demonstrate unequivocal changes in fat distribution (we note that skin-fold thickness is a quite distant surrogate of central ie intra-abdominal fat), and we are unable to comment with any confidence on whether dietary changes could have been mediated, in part, by changes in body fat.

Blood pressure:

About 50% of patients with NIDDM are hypertensive. Hypertension may be due to increased total peripheral resistance or increase in cardiac output or both (Vander et al., 1994 b). About 95% of people with hypertension have essential (or primary) hypertension (Ross, 1990; Durrington, 1995).

Istfan et al (1992) have reported that obese hypertensive subjects had greater insulin resistance than obese non-hypertensive subjects. It is thought that the relationship between primary hypertension and insulin resistance is independent of obesity (Pollare et al., 1990), but the nature of the association of hypertension with obesity or the insulin resistance syndrome is obscure.

In this study, the HMUFA group demonstrated a significant reduction of SBP, DBP, and MAP during 12 months, compared with the Control group. Blood pressure reduction in those on the high-MUFA diet did not seem to be caused by weight loss or by reduction in the sodium, potassium or calcium intake (Rasmussen et al., 1993) or by changes in medication.

Rasmussen et al., (1993) also reported reduction of SBP and DBP in participants who followed the HMUFA diet, in which olive oil and almonds were major sources of monounsaturated fatty acids.

Systolic blood pressure was significantly correlated (pairwise correlation) with waist ($r = 0.39$, $P=0.009$) and hip circumference ($r = 0.41$, $P=0.006$) and with indices of poor diabetic control [NEFA ($r = 0.38$, $P=0.012$), VLDL-TG ($r = 0.32$, $P=0.036$) and inversely correlated with glucose uptake with the euglycemic clamp ($r = -0.41$, $P=0.050$)], and with fat intake in the diet ($r = -50$, $P=0.001$), and monounsaturated fat (%) intake ($r = -0.42$, $P=0.005$). Thus people with abdominal obesity, high insulin resistance (and the dyslipidemia characteristic of poor diabetic control) and low monounsaturated fat intake were more likely to have a higher systolic blood pressure.

Diastolic blood pressure was also significantly correlated with waist circumference ($r = 0.33$, $P=0.029$), BMI ($r = 0.31$, $P=0.043$), skinfold measurements at biceps (r

= 0.38, P=0.011) and suprailiac (r = 0.33, P=0.030) site, NEFA (r = 0.47, P=0.001), VLDL-TG (r = 0.31, P=0.049), CETP% (r = 0.42, P=0.005), saturated fat (%) intake (r = 0.31, P=0.0473), and inversely correlated with monounsaturated fat (%) intake (r = -0.39, P=0.010). Again, people who were fatter, dyslipidemic, and with a low monounsaturated fat intake were likely to have a higher diastolic blood pressure.

Mean arterial pressure was (not surprisingly) correlated with similar variables: waist (r = 0.39, P=0.010) and hip circumference (r = 0.38, P=0.012), skinfold thickness in biceps (r = 0.33, P=0.029) and suprailiac areas (r = 0.32, P=0.038), NEFA (r = 0.45, P=0.002), VLDL-TG (r = 0.34, P=0.028), CETP% (r = 0.38, P=0.013), and inversely correlated with fat intake in the diet (r = -0.42, P=0.006) and monounsaturated fat intake (r = -0.44, P=0.004) (both expressed as a % of energy intake).

The effect of dietary fats on blood pressure could be related to changes in the fluidity of cell membranes depending on the fatty acid composition of the membrane itself (Trevisan et al., 1990). Membrane fluidity indeed is affected by the fatty acid composition of the diet, being reduced when the content of saturated fatty acids is higher, and increased when the degree of unsaturation is higher (Trevisan et al., 1990). Membrane fluidity in turn might be a factor regulating ion transport system across the cell membrane, whereas changes of such systems have been related to the genesis of hypertension (Pagnan et al., 1989).

Our study suggests that consuming diet high in monounsaturated fat can decrease significantly the blood pressure in subjects with NIDDM. The mechanism of course is uncertain; whether, for instance, the n-3 content or the n-9 oleic acid is responsible, or whether regional fat loss is responsible, and if so what the

mechanism is, is not clear. One should also remember that monounsaturated fat intake is not entirely independent of saturated fat intake; although a statistical relationship between saturated fat intake and blood pressure was not shown, it is possible that saturated fat intake has a causal effect in inducing high blood pressure. However, there is little direct evidence to support this supposition.

Lipids:

In diabetes a main cause of morbidity and the major cause of premature mortality is atherosclerosis, in which the disordered lipoprotein metabolism of diabetes almost certainly has a leading role (Durrington, 1995).

It has shown in previous studies that plasma NEFA concentrations are higher than normal in patients with NIDDM (Reaven, 1995; Park, 1993). Three months into the study there was a significant reduction of NEFA in HMUFA diet group compared to the Control group. Within the HMUFA group also there was a significant reduction of IDL-TG and IDL-FC.

By 12 months a number of plasma lipid changes had occurred. In the group on the HMUFA diet had reduced plasma-TG, NEFA, VLDL-TG, IDL-FC, and CETP activity, and increased HDL-C and HDL-Apo A.

There is evidence that the dietary approach to patients with NIDDM should attempt to decrease plasma very-low-density lipoprotein (VLDL) triglyceride and increase high-density lipoprotein (HDL) concentration, as these two factors influence the risk of developing macrovascular disease (Reaven, 1988a). Within the diabetic population the risk of atherosclerosis has shown to be related to serum lipid levels, both serum cholesterol and in particular serum triglyceride (Durrington, 1995).

It has been shown in many studies that dietary saturated fatty acids is a risk factor for the development of coronary heart disease; the mechanism is subject to debate, but a major factor may be a decrease in the protective HDL cholesterol (Stanton 1992). Animal studies have suggested that a diet rich in cholesterol and saturated fat (an atherogenic diet) enhances the plasma CETP activity (Stein et al, 1990, Quinet et al, 1990), and leads to decreased levels of HDL-C (Grundy and Denke, 1990). Since the major nutritional recommendations for NIDDM include reducing saturated fat and cholesterol intake in the diet (because of the consequent high incidence and prevalence of cardiovascular disease (ADA, 1993), the decreased intake of saturated fat and increased intake of monounsaturated fat in the HMUFA diet could reduce the risk of heart disease in NIDDM patients.

This study supports the notion that increased intake of monounsaturated fat in the diet can reduce serum triglyceride and increase HDL-C, as found by others as well (Jenkins et al., 1989; Garg, 1994; Campbell et al., 1994).

In contrast, some investigators (Berry et al., 1992; Wahrburg et al., 1992) have not found significant increases in HDL-C following HMUFA diet. This could be explained by short duration of their studies; in our study there were no significant changes in the first 3 months in HDL-C for instance, but changes were noticed at 12 months.

LDL-C was reduced in both groups during the study (Control: from 3.40 mmol/L at 0 time to 3.10 mmol/L at 12 months; HMUFA: from 3.45 mmol/L to 2.97 mmol/L at 12 months), but in contrast to previous studies (Stanton, 1992; Lichtenstein et al., 1993; Sarkkinen et al., 1994), there was no significant differences between the diet groups during the study.

Blood glucose and related variables:

FBG decreased significantly in the HMUFA group during 12 months, compared with the Control diet group. FBG was correlated with indices of dyslipidemia [plasma-TG (r = 0.34, P=0.027), CETP activity (r = 0.47, P=0.031), NEFA (r = 0.67, P=0.000)] and obesity [subscapular measurement (r = 0.55, P=0.005), body fat % (r = 0.42, P=0.039), skinfold-sum (r = 0.45, P=0.016), body density (r = -0.42, P=0.042)], glucose uptake in euglycemic clamp studies, ie insulin sensitivity (r = -0.50, P=0.017), dietary fat intake (r = -0.45, P=0.003), and monounsaturated fat (g) intake (r = -0.32, P=0.040).

In summary, subjects who had a high fasting blood glucose tended to be fat, dyslipidemic and resistance to the action of insulin with a low monounsaturated fat intake.

Garg et al. (1988), Rasmussen et al. (1993), Lerman-Garber et al. (1994), and Campbell et al. (1994) have also described a reduction of FBG following a HMUFA diet. The duration of these studies were 2 to 4 weeks, in contrast to our 12 months study.

It has been proposed in previous studies that in individuals with NIDDM, the greater the increase in plasma NEFA concentration, the higher is the plasma glucose concentrations which compromises the function of beta-cell secretion (Golay et al., 1987; Reaven, 1995). In this study both NEFA and plasma glucose decreased significantly in HMUFA group, but the mechanism behind this changes and the role of monounsaturated fat in this mechanism needs more investigation.

In the some published studies (Ciardullo et al., 1993; Garg et al., 1988) plasma insulin concentration felt significantly with a HMUFA diet in 2 to 4 weeks. In the

current study plasma insulin level did not change significantly during 12 months and remained in the reference range in both groups (normal range= <20 mIU/L). Garg et al., (1992), in their study of the effects of a HMUFA diet on NIDDM patients, did not report any changes on plasma insulin. The reasons for this lack of change in plasma insulin are quite unclear.

Plasma C-Peptide did not change significantly during 12 months and remained above the normal range in both groups (normal range = 0.5-3.0 ug/L). It was correlated with plasma-cholesterol (r = 0.46, P=0.024), weight (r = 0.37, P=0.014), waist circumference (r = 0.39, P=0.010), hip circumference (r = 0.43, P=0.004), and suprailiac skin-fold thickness (r = 0.31, P=0.041), dietary protein (g) intake (r = 0.58, P=0.003), simple CHO intake (r = 0.47, P=0.022), fat (g) intake (r = -0.42, P=0.043), and monounsaturated fat (g) intake (r = -0.42, P=0.040) were also correlated. Subjects with a high fasting C-Peptide tended to be overweight with a high plasma cholesterol and a high dietary intake of CHO and protein and a low dietary intake of monounsaturated fat.

Although mean HbA1c was increased in the Control group from 7.8% to 8.0% and reduced in the HMUFA group from 8.5% to 7.9% during 12 months, these were not statistically significant changes. Glucose control in the Control group was 'good' and in the HMUFA group was 'excellent', at 12 months (reference range noted in chapter 3). HbA1c was correlated with plasma-TG (r = 0.36, P=0.019), NEFA (r = 0.64, P=0.001), skinfold thickness at triceps (r = 0.41, P=0.049), and subscapular area (r = 0.49, P=0.015), and dietary fat intake (r = -0.32, P=0.036). A person with high HbA1c was more likely, therefore, to be dyslipidemic, obese, and to have a low fat intake.

Insulin resistance:

Insulin resistance both precedes and predicts the occurrence of diabetes (Vessby, 1995), although 50% of insulin-resistant individuals never develop overt NIDDM. This suggests that insulin resistance by itself is not sufficient to cause NIDDM and that a second defect is required (Ogawa et al., 1992; Weir and Leahy, 1994; Reaven, 1995). It is generally considered the second defect is a deficiency in insulin production.

Insulin resistance has been shown to be influenced by environmental factors (Walker and Alberti, 1993; Simopoulos, 1994a), and diet has been considered as the major environmental factor (Storlien et al., 1991). In this study, direct measurements of insulin resistance by the euglycemic glucose clamp method showed that the increased intake of monounsaturated fat and reduction of saturated fat intake lead to decreased insulin resistance and increased insulin sensitivity in HMUFA group.

Glucose uptake by the euglycemic clamp was correlated with subscapular measurements ($r = -0.46$, $P=0.029$), FBG ($r = -0.49$, $P=0.017$), plasma-C ($r = -0.51$, $P=0.013$), and CETP activity ($r = -0.59$, $P=0.004$). Insulin resistant individuals therefore tended to have dyslipidemia, and to have a slightly high fasting blood glucose.

Using pairwise correlation, there were significant correlations between glucose uptake and the quality of dietary fat (Poly:Sat:ratio ($r=0.51$, $P=0.013$), and Mono:Sat:ratio ($r=0.46$, $P=0.028$). Other studies also have demonstrated a reduction in insulin resistance, as a result of an increased intake of monounsaturated fat in the diet (Riccardi and Rivellese, 1991; Ciardullo et al., 1993).

In regard to metabolic abnormalities of insulin resistance, it has been mentioned in previous studies that increased levels of VLDL-TG (Zavaroni, 1993; Reaven, 1988; Alford, 1996), decrease levels of HDL cholesterol (Stalder et al., 1981; Zavaroni et al., 1985; Laakso, 1993; Alford, 1996), and hypertension (Daly and Landsberg, 1991; Reaven, 1991; Laakso, 1993; Nosadini et al., 1993; Shafrir 1993; Nosadini et al., 1994; Alford, 1996) can worsen the insulin resistance. In this study, VLDL-TG, SBP, DBP, and MAP were reduced significantly and HDL-C were increased significantly in HMUFA group.

There is growing evidence that the development of insulin resistance in NIDDM may be closely related to abdominal adiposity (Kissebah et al., 1982; Krotkiewski et al., 1983; Ducimetiere et al., 1986; Park, 1993; Kohrt et al, 1993; Banerji et al., 1995; Walker, 1995). There were significant reductions in all skinfold measurements and percent body fat in the HMUFA group, and this may have been the cause of increased insulin sensitivity.

In the Control group there was no improvement in insulin resistance as glucose uptake continued to decline over the 12 months of the study (0 time = 130 $mg.m^{-2}.min^{-1}$, 3 months = 123 $mg.m^{-2}.min^{-1}$, 12 months = 91 $mg.m^{-2}.min^{-1}$). This worsening of insulin resistance has also been reported by others in studies on humans (Parillo et al., 1990; Borkman et al., 1991) and animals (Kraegen, 1986; Storlien et al., 1986; Storlien et al., 1991; Ikemoto et al., 1995).

Medication intake, cigarette smoking and alcohol intake did not significantly change during the 12 months, and could not have affected the changes in insulin resistance.

Indirect calorimetry:

Studies using indirect calorimetry have been interpreted as showing that the peripheral insulin resistance of NIDDM may preferentially affect nonoxidative glucose metabolism rather than oxidative glucose metabolism (Moller and Flier, 1991). In this study however there were no significant changes in oxidative or non-oxidative glucose metabolism.

Diet acceptability and feasibility:

Dietary therapy for persons with diabetes is based on an assessment of usual eating habits, life-style factors, and outcome goals. A major component of the assessment is to determine what the individual is able and willing to do. The treatment plan is then designed to meet desired outcomes (Coulston, 1994a).

The comments made by participants in the questionnaire indicated that a number of patients in the Control group were bored with their diet and had a problem in that they had different food from their family.

By contrast, the HMUFA group, although some of them had an initial problem with their family in accepting the new changes in their diet, but in the long term felt happier and healthier. Some even mentioned that they were less sick this year than ever before. Most implemented this diet for the rest of their family as well.

This study showed that NIDDM patients who followed the HMUFA diet in general were more satisfied with the diet, found it more convenient for their family, found it tastier, and was easier to prepare, when compared with their previous diet recommended for NIDDM patients. Walker et al., (1995) has also reported that patients who followed the HMUFA diet felt more active and energetic.

Many studies have shown that most people with NIDDM do not achieve the treatment goals recommended by the American Diabetes Association (Consensus Statement, 1995). Long-term weight loss for instance, is beyond the capacity of most. A HMUFA diet seems more practical than a "low fat high carbohydrate" diet, more beneficial for NIDDM patients to follow in a long time period, and more likely to be followed.

CHAPTER SIX

CONCLUSION

In a country of 17 million people, diabetes ranks fifth after heart disease, stroke, musculo-skeletal disease and cancer as a cause of death. If grouped together with vascular disease, the combination is the most common cause of death in Australia. A similar pattern has been in other countries - a worldwide explosion in the frequency of diabetes and the morbidity resulting from its complications. At present, 45.8% of the direct annual costs of diabetes are spent in hospital care with a further 22.6% in nursing home care, leaving only some 22% of diabetes costs directed towards medical care, health personnel, drugs, etc. At least two-thirds of this cost is related to NIDDM and its complications (Turtle et al., 1994).

The risk of developing NIDDM is associated with diet and can be controlled to a large extend by food choices (Nettleton, 1995). Therefore, dietary treatment has been accepted as an integral part of the management of NIDDM (Milne et al., 1994).

The approach to the dietary treatment of patients with diabetes mellitus has changed in the last decade. Diabetologists are becoming increasingly more aware that the diabetic diet should not only help improve blood glucose control but also help prevent cardiovascular disease (Riccardi and Rivellese, 1991). In diabetes a main cause of morbidity and the major cause of premature mortality is atherosclerosis, in which disordered lipoprotein metabolism, particularly serum triglyceride has a leading role (Durrington, 1995).

This study dealt with an important area on which public health advice is unclear; what kind and level of fat consumption should be encouraged in the NIDDM diet, and can a change in patterns of fat consumption bring about changes in metabolic control, insulin resistance, and insulin resistance syndrome?

A diet based almost exclusively on vegetable products does not appear feasible for most people in western countries. A moderate increase in the consumption of fibre-rich foods, especially legumes, vegetables, and fruits (increasing the daily fibre intake up to 40g) combined with a preferential use of unsaturated fat seems likely to be more easily accepted. A diet low in saturated fat and cholesterol and rich in vegetables and olive oil resembles the type of diet used in the Mediterranean region that is becoming increasingly popular in many western countries (Riccardi and Rivellese, 1991), and our study suggests that it is more acceptable and effective than the standard low fat high carbohydrate diet currently recommended.

Some diabetologists are now concerned about recommending a high carbohydrate diet to patients with NIDDM because of the potentially harmful effects of such diets on plasma lipids. Increasing plasma triglycerides, VLDL cholesterol and triglyceride levels and lowering of HDL cholesterol concentrations are considered undesirable changes; potentially atherogenic.

Considering that the low fat high carbohydrate diet tends to result in metabolic changes associated with atherosclerosis and since adherence is poor, it seems only feasible that another nutrient replace some of the complex carbohydrate. Monounsaturated fat has been suggested as the alternative. Research has demonstrated that a diet containing MUFA tends to meet the goals associated with the treatment of NIDDM (Garg et al., 1988; Mensink et al., 1989 Bonanome et al., 1991; Mata et al., 1992; Campbell et al., 1994., Lerman-Garber et al., 1994).

Cardiovascular disease:

Cardiovascular disease is an established fact as the leading cause of morbidity and mortality among patients with NIDDM. Moreover, such people are often obese, suffer from hypertension as well as dyslipidaemia and are insulin resistant.

Data from a number of epidemiological studies support a strong association between hypertension and NIDDM. Furthermore, there is epidemiological evidence to suggest that insulin resistance is an important factor in the development of hypertension in both obese and lean individuals. However, the relationship between hypertension and insulin resistance would appear to be dependent on ethnicity as well as genetic or environmental factors.

The benefits of blood pressure reduction on stroke rate, congestive heart failure and renal disease are widely known. Blood pressure control is particularly important in diabetic people, since a small increase in blood pressure can accelerate microvascular and macrovascular complications.

Dyslipidaemia is frequently found in people with NIDDM, as demonstrated by a number of epidemiological studies in chapter two. Furthermore, the typical dyslipidemia of NIDDM - raised triglycerides and low HDL cholesterol - is associated with the presence of insulin resistance.

Insulin resistance both precedes and predicts the occurrence of diabetes (Vessby, 1995). Insulin resistance provides the pathophysiological basis for the increased risk of cardiovascular morbidity in NIDDM (Nosadini et al., 1993).

In relation to genetic factors, since there is not yet any way of influencing the genetic components of NIDDM, research on intervention has focused on environmental aspects. Diet is one of the main environmental factors which plays a vital role in treatment of NIDDM.

In this study, the benefits of dietary modification are clearly apparent in the management of NIDDM, since modification of fat intake for NIDDM has proved to influence the significant reduction of the systolic and diastolic blood pressure. Although the primary reason for this reduction is not clear, but since the insulin sensitivity has improved significantly following the modified fat diet, and there has been a positive correlation between insulin resistance and hypertension, therefore, that could explain the mechanism of blood pressure reduction in NIDDM (Figure 6.1).

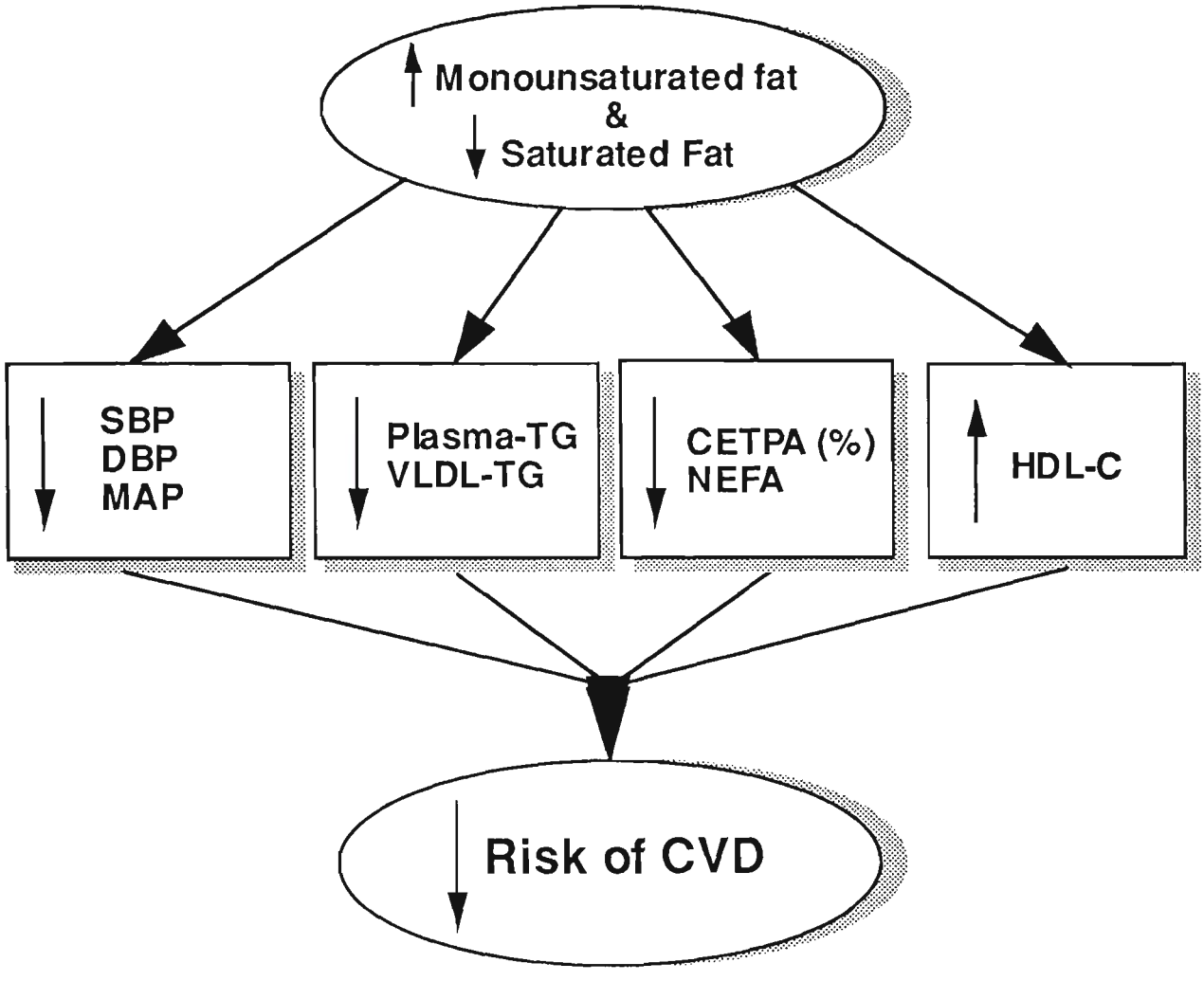

Figure 6.1: The effects of modified fat diet on risk factors for cardiovascular disease.

Diet acceptability / achievability:

Nutrition is a changing field, and continuing evaluation and education are essential for people with diabetes to incorporate these changes in their management programs.

Nutrition therapy for person with diabetes is based on an assessment of usual eating habits, life-style factors, and outcome goals. A major component of the assessment is to determine what the individual is able and willing to do. The treatment plan is then designed to meet desired outcomes, which can be evaluated by self-

management reports and some predict evaluation like: plasma lipid concentrations, and blood pressure. (Coulston, 1994a).

One of the primary reason that dietary therapy is difficult for people with diabetes is that adherence to any diet is difficult. This is particularly true if the diet is not satisfying or if the diet differs from that of the general population (Bantle, 1992).

Despite the metabolic problems associated with the currently recommended diet for NIDDM, there are issues of acceptability and long term adherence. In this study, we found poor adherence to the currently recommended diet for NIDDM, as participants on the High-CHO low fat diet were unable to maintain this diet for one year. On the contrary, the HMUFA diet group tolerated (indeed, adopted) the monounsaturated diet very well. On the whole participants assigned to the diet with liberal fat enjoyed their diet, and with objective measures of palatability and acceptability we have quantitative evidence that the HMUFA diet is seen preferable and maintained much better by a higher proportion of subjects than those given the low fat high carbohydrate diet.

This study suggests that it is essential the meal plan, education, and counselling program be individualized for the person with diabetes, with the basic goals of meal planning in mind, the individual's plan needs to be realistic and provide as much flexibility as possible, allowing integration of therapeutic measures into individual's life-style educators must utilize techniques and written materials appropriate for the patients and their family. The same time, appropriate traditional, ethnic, and cultural foods can be encouraged.

This study showed that a nutritional program based on an intake of HMUFA which reflects the "normal" Australian diet, within a diet pattern with Mediterranean and Asian characteristics, can improve the condition of NIDDM patients.

The relationships between the variables under study are shown in figure 6.2. The data on which this figure is based relate to associations; other studies are required to establish causal relationships.

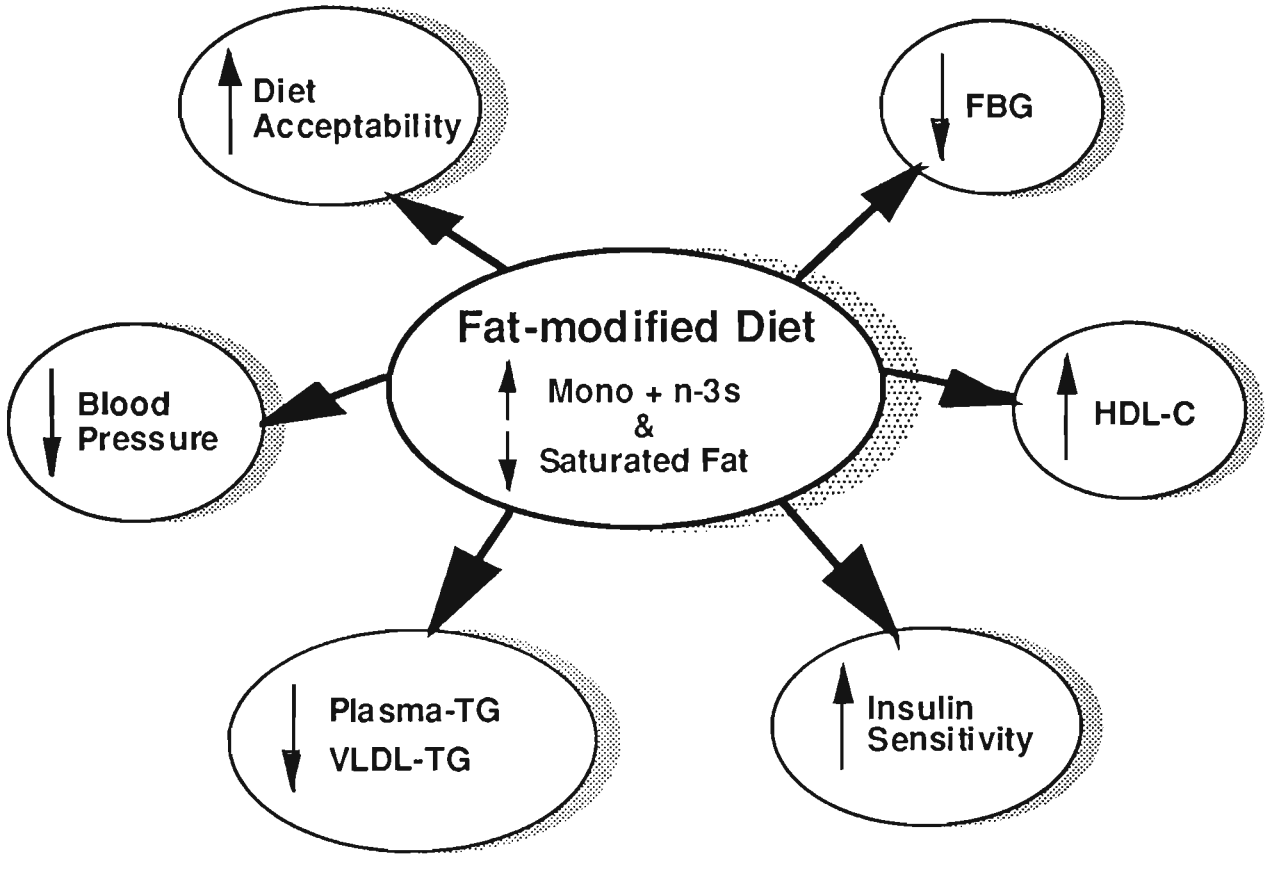

figure 6.2: The relationships between modified fat diet and different variables.

In conclusion, this study showed that increasing monounsaturated fat intake (especially from canola which also contains n-3 fatty acids) and decreasing the saturated fat intake in the NIDDM diet can be both effective and practicable. The way appears clear for the trialing of health promotion techniques to apply these findings more widely, and to evaluate wider implementation.

Further Investigation

* Since obesity is well recognised as a major cardiovascular risk factor and it is thought to contribute to the development of insulin resistance and NIDDM, further investigation on the effect of modified fat diet, in the reduction weight bases, on the insulin resistance, lipid and lipoproteins and glycemic index would be essential.

* In this study, the diet intake was based on the multiple four days food record. It would be interesting to find out the changes in the food intake on the red blood cells, or look at the fatty acid composition in the serum cholesterol esters.

* Recently studies have been done on the changes in Leptin in NIDDM. Therefore, it is interesting to see if the changes in the diet intake could affect the level of Leptin in the blood.

* This study was carried out for the duration of one year. There is a need to study these people for longer period, to find out about the effect of diet on different metabolic parameters and also the adherence to the diet.

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APENDIX I:

Informations, letters, and medication intakes

Dear .....

We are members of the Medical Research Unit (University of Wollongong and Illawarra Area Health Service) and we are undertaking a study of the effect of diet in controlling diabetes mellitus. We are interested in the form of diabetes that develops in adults, and we understand from the Diabetes Education Centre of the Illawarra Area Health Service you are likely either to have this or to have a tendency to this form of diabetes. If so you will know that diet is an important part of the treatment, and that diet will often involve changing the kind and amount of fat eaten.

The kind of study we want to undertake involves testing during a morning to see how severe your diabetes (or your diabetic tendency) is, and to carry out a check on a number of factors that might be associated with a diabetic tendency (your body shape, your blood cholesterol and blood fats) and then seeing how you respond either to your usual diet recommended for diabetes or to a modified diet. The study would continue for a year, with a check on your diabetes or diabetic tendency at the beginning and after three and twelve months and checks on your diet from time to time.

We hope you are able to help in this study. If you are not interested in taking part or if you would like more details please ring Mrs Elaine Knight (042) 266 594, secretary to the Medical Research Unit. If we do not hear from you, we shall ring to ask if you are interested and offer further details, and (if you are interested) ask permission to contact your doctor. We would not proceed any further without consulting your doctor, who has to be well aware of and agree with any dietary study. Do not feel obliged to take part in the study if you feel you would not like to; your treatment and the welcome you receive at the Diabetes Education Centre will not in any way be affected if you do not want to take part (or if you change your mind and withdraw).

The study would start in July-August.

Yours sincerely

G D Calvert
Professor of Medicine and Public Health

Ms Mahnaz Fanaian
Registered Nurse
and graduate student

UNIVERSITY OF WOLLONGONG

THE RELATIONSHIP BETWEEN INSULIN ACTION AND DIET IN NON-INSULIN DEPENDENT DIABETES MELLITUS

INFORMATION AND CONSENT FORM

Investigators: Professor Dennis Calvert, Professor of Medicine and Public Health, Medical Research Unit, University of Wollongong and Illawarra Area Health Service.

Ms Mahnaz Fanaian, student enrolled for Doctor of Philosophy (PhD) in the University of Wollongong, registered nurse, specialist in coronary care/intensive care.

WHY IS THE STUDY BEING DONE?

Non-insulin dependent diabetes mellitus is the most common form of diabetes. Most of those affected are in middle age or older, and most of those that have this problem are overweight.

Recommended initial treatment is by dietary change. A number of dietary changes that have been shown to be effective in short studies with committed people have proved much less effective in clinical practice. One reason is that many people find it very hard or impossible to lose weight, and another is that many people are unable to change radically the eating habits of a lifetime.

We would like to study the effect of one of the following diets which you will be assigned:

1. a diet along the lines recommended by the American Diabetes Association (high in complex carbohydrates, low in fat).

2. a diet similar to your usual diet, keeping the total fat within your usual range but substituting foods which contain monounsaturated fat (canola oil and olive oil are two fats which are largely monounsaturated).

The results of this study will be published in a journal so that they can be used, we hope, to manage non-insulin dependent diabetes better.

WHO CAN VOLUNTEER TO TAKE PART IN THE STUDY?

People who have been told that they have diabetes mellitus and who do not require insulin treatment for the treatment of diabetes (and who prove to have non-insulin dependent diabetes mellitus) are invited to join the study. People taking part must also be reasonably well and their normal diet must be representative of most Australians. We offer a health check and a diet analysis to people offering to take part.

WHAT HAPPENS IF ANYONE DECIDES NOT TO TAKE PART?

Participation in the study is entirely voluntary and people may not wish to be involved or may discontinue involvement at any time. A decision not to take part or to drop out from the study will not affect anyone's treatment by the hospital or medical or diabetic services or by the University.

WHAT DOES THE STUDY INVOLVE?

The study lasts for one year. It involves following one of the above mentioned diets. Your diet will be individually prescribed by a dietitian working in consultation with you, to get a diet that is attractive and sustainable. You will need to spend some time practising dietary measurements and recording your diet accurately.

The order in which you take the diets is decided by chance. Your diet will be recorded by yourself in a four-day food diary before the study starts, then once a month for 12 months. We will ask you questions about your acceptance of each dietary program. You will try and maintain a constant weight during this period.

At the beginning of the study, after three months and again after 12 months you will be asked to have some measurements taken (waist and hip girth, thickness of skinfolds at several sites).

We will then assess insulin resistance. This is performed by giving two separate infusions through the catheter, both glucose and insulin. Blood samples for glucose and insulin measurements and blood fats including cholesterol, after a 10-12 hour overnight fast are then taken through the other catheter, and the amount of "insulin resistance" can be calculated from the levels. The assessment of insulin resistance takes about two and a half hours. A meal is given at the end of the study. The blood sugar level is maintained at a normal level at all times.

WHAT ARE THE RISKS OR DISCOMFORTS OF THE STUDY?

The potential hazards of participation in this study are minimal.

Occasionally some irritation occurs at the site of the plastic catheters used for infusions and blood sampling, or there may be clotting of the vein or some bruising; however, these would usually be temporary and expected to resolve completely. In some volunteers a mildly low blood glucose level may occur transiently following the insulin injection; this is most unlikely in people who have non-insulin dependent diabetes, and in any case will be monitored by a doctor to ensure that it is not harmful.

FURTHER INFORMATION

If you have any further questions about this study, Professor Dennis Calvert from the Medical Research Unit, Illawarra Regional Hospital (Wollongong) (telephone 042-266 594) will be happy to discuss them with you.
If you have any complaints you may contact the secretary of the Human Research Ethics Committee, University of Wollongong, Ms Karen McRae, on phone (042)-214 457.

UNIVERSITY OF WOLLONGONG

THE RELATIONSHIP BETWEEN INSULIN ACTION AND DIET IN NON-INSULIN DEPENDENT DIABETES MELLITUS

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WHAT HAPPENS IF ANYONE DECIDES NOT TO TAKE PART?

Participation in the study is entirely voluntary and people may not wish to be involved or may discontinue involvement at any time. A decision not to take part or to drop out from the study will not affect anyone's treatment by the hospital or medical or diabetic services or by the University.

WHAT DOES THE STUDY INVOLVE?

The study lasts for one year. It involves following one of the above mentioned diets. Your diet will be individually prescribed by a dietitian working in consultation with you, to get a diet that is attractive and sustainable. You will need to spend some time practising dietary measurements and recording your diet accurately.

The order in which you take the diets is decided by chance. Your diet will be recorded by yourself in a four-day food diary before the study starts, then once a month for 12 months. We will ask you questions about your acceptance of each dietary program. You will try and maintain a constant weight during this period.

At the beginning of the study you will be asked to have some measurements taken (waist and hip girth, thickness of skinfolds at several sites) we will then take a blood sample for the measurement of blood glucose, insulin and blood fats including cholesterol, after a 10-12 hour overnight fast.

After three months volunteers will attend the Illawarra Regional Hospital while fasting. A blood sample is taken for repeat estimation of blood glucose, insulin and blood fats.

WHAT ARE THE RISKS OR DISCOMFORTS OF THE STUDY?

The potential hazards of participation in this study are minimal.

FURTHER INFORMATION

If you have any further questions about this study, Professor Dennis Calvert from the Medical Research Unit, Illawarra Regional Hospital (Wollongong) (telephone 042-266 594) will be happy to discuss them with you.

If you have any complaints you may contact the secretary of the Human Research Ethics Committee, University of Wollongong, Ms Karen McRae, on phone (042)-214 457.

University of Wollongong

Human Research Ethics Committee

CONSENT FORM

Measurement of Insulin Sensitivity

Ms Mahnaz FANAIAN

This research project is being conducted as part of a Doctor of Philosophy supervised by Professor Dennis Calvert in the department of Medical Research Unitat the University of Wollongong.

Information relating to this study is detailed in the attached information sheet.

You are free to withdraw from the research at anytime without penalty.

If you have any enquiries regarding the conduct of the research please contact the Secretary of the University of Wollongong Human Research Ethics Committee on (042) 213079.

I understand that the data collected will be used to study the relationship between insulin action and diet in non-insulin dependent diabetes mellitus and I consent for the data to be used in that manner.

If you wish to take part in this research please sign below:

..../...../.....

Dear Dr xxx
Re: Mr xxxxxxxx
DOB xx xx xx

A group of us at the Medical Research Unit (IAHS and University of Wollongong) together with staff of the Diabetes Education Centre are engaged in a program of research into the causes and management of non-insulin dependent diabetes mellitus and the insulin resistance syndrome (abdominal obesity, insulin resistance, hyperglycemia, dyslipidemia including hypertriglyceridemia and low HDL cholesterol and high blood pressure).

I am writing to you to tell you about a current study and to ask if the enrolment of **Mr xxxxx**, a patient of yours, in that study would give rise to any problems.

We wish to study the effectiveness and acceptability of the "Mediterranean" type of diet, a variant of the southern European dietary pattern used in the Lyon Heart Study and the Cretan limb of the Seven Countries heart study, and compare it with the standard diet currently prescribed for people with non-insulin-dependent diabetes mellitus. To this end we wish to invite 25 pairs of people with non-insulin-dependent diabetes, each pair matched for age, sex and body mass index, to take part in a comparative diet study for one year. The Mediterranean arm would have a diet which is rich in green and root vegetables, low in saturated fat but relatively enriched in canola and olive oil or margarine, and which contains white meat and fish but little or no red meat. The usual diet arm would have the diet that the participants have been counselled on at the Diabetes Education Centre, ie one rich in complex carbohydrate and low in fat especially saturated fat. Measurements include dietary analysis, fasting blood lipids, insulin and glucose, and a measure of insulin resistance by a technique known as the euglycemic clamp, in which the ability of insulin to move glucose out of the blood stream is measured. Participants should be otherwise healthy and should understand clearly what is proposed and what the implications for them are. Patients should benefit in that they will be encouraged to adhere to a diet (either the standard diet they have been prescribed or the Mediterranean one) and in the process I expect they will learn a great deal about the control of diabetes mellitus.

Mr zzzzz has been approached through the Diabetes Education Centre and has expressed interest in joining the study. **He** has given your name as **his** family doctor, and I am writing to check that there is no reason why **he** should not enrol in the study.

I would be very much obliged if you could let me know if you see any difficulties in enrolling **Mr zzzzz** into this study, or if there is anything we should know about this course of action. If we don't hear from you we will assume that there are no problems and, if **Mr zzzzz** is still willing and is not excluded on other grounds, we shall enrol **him** into the study.

Yours sincerely,

Dennis Calvert MD FRACP
Professor of Medicine and Public Health

Dear Dr .....
re: «patient»
«patient» has been enrolled in a study we have running on the effects of two diet regimens in non-insulin dependent (Type II) diabetes since ..... The diets differ considerably; both have been recommended. I understand «patient» has consulted you about aspects of «hisher» diabetes. At the beginning of the study we wrote to all doctors nominated by patients as their doctor to ask if there were any problems with the patient's inclusion in the study. Because there may have been changes or because the doctors looking after the study participants now may be different from those looking after them before the study began, I thought it best that I write again to bring you up to date with the study.

We enrolled 50 patients, half randomly allocated a study group on the standard low-fat high-complex carbohydrate diet (this is the currently recommended diet in the standard guidelines) and half in a fat-modified diet (a "Mediterranean" diet) in which monounsaturated fat (canola and olive oil) is substituted for other fat, also recommended by many. An experienced dietitian, Ms Joanne Szilasi, is running the dietary side of the study.

The monounsaturated fat-rich diet is not high in kilojoules, as mono-rich foods are substituted for other foods, usually meats. Meat serves, especially red meat, are kept to a minimum and chicken and fish are substituted. Peanuts (monounsaturated fat) may be substituted for biscuits (saturated fat) etc. Butter, cream, pies, cakes, biscuits, sausages, processed meats, chocolates are avoided, and other foods often containing monounsaturated fat are substituted.

We are monitoring insulin resistance, glucose disposal, glycosylated hemoglobin, weight and indices of adiposity, and other data.

So far, six months into the study, most patients have done well in achieving their dietary goals, but some still have a way to go. We are learning about the difficulties faced by free-living patients in Australia in achieving dietary change. Metabolic indices are changing in the expected directions in both groups, but so far the changes are (on the whole) not significant or of borderline significance on a simple statistical test. The one significant change so far is that patients on the modified fat ("Mediterranean") diet have had a significant improvement in insulin sensitivity (as measured by glucose uptake using an insulin clamp technique) when considered as a group.
The study has six months to run before we sum the results. We will of course be letting all doctors (nominated by participants) know about the findings. In the meantime we would be very pleased if you could let me know of any problems arising in patient management. Patients may, of course, need to have their treatment altered for all sorts of reasons during this year. It would be very helpful though, if there are no clinical contraindications, for patients to continue on their assigned dietary program for the duration of the trial. We would appreciate this, as the trial is constructed with minimum enrolled numbers that can reliably be expected to produce measurable (and worthwhile) effects. I would welcome any discussion or questions on the study.

Yours sincerely

G D Calvert Professor of Medicine and Public Health

Dear

Re:
DOB:

I recently wrote to you about a nutritional research program this Unit is conducting into non-insulin dependent diabetes and the place of monounsaturated fat in their diet and asking if there were any problems if took part. is now taking part in the research study.

The following are the results of some recent tests on :

TEST	VALUE	NORMAL OR IDEAL RANGE
Glucose (fasting)		3.8-7.8 mmol/L
Total cholesterol		<5.5 mmol/L
Triglycerides (fasting)		<2.0 mmol/L
HDL cholesterol		>0.9 mmol/L
Blood Pressure		
Weight	kg	
Height	cm	

Yours sincerely

Dennis Calvert MD FRACP
Professor of Medicine and Public Health

Ms Mahnaz Fanaian
Registered Nurse
and graduate student

DEMOGRAPHIC 0 TIME

CODE: _____ Date: _____

Surname: Name: Sex: Age:

Date of birth: Marital Status: Telephone No:

Address:

_____

Name and address of family doctor / specialist:

_____

Medical History: _____ Yes _____ No

If yes, specify:_____

_____

Duration of Diabetes: _____ Years or _____Months

Medication: _____ Yes _____ No

Hypoglycaemic drug: _____ Yes _____ No

Other medication: _____ Yes _____ No

If yes, specify: _____

_____

History of diabetes in your family: _____ Yes _____ No

If yes, specify: _____

_____

Do you smoke? _____ Yes _____ No

_____ previously smoker: _____ per day _____ stopped date: _____

_____ currently smoking: _____ per day

Do you do any regular exercises? _____ Yes _____ No

If yes, specify:

_____

_____

Do you drink alcohol? _____ Yes _____ No

_____ previously drinker: _____ per day _____ stopped date: _____

_____ currently drinking: _____ per day

DEMOGRAPHIC 3 MONTHS

CODE: _____ Date: _____

Surname: _____ Name: _____ Sex: _____ Age: _____

Date of Birth: _____ **Marital Status:** _____ **Telephone No:** _____

Is your address the same as before? _____ Yes _____ No

If No, please specify: _____

Is your family doctor / specialist the same as before? _____ Yes _____ No

If No, please specify: _____

Have you had any health problem in the last 3 months? ____ Yes ____ No

If yes, specify:_____

Do you take any medication? _____ Yes _____ No

If yes, please specify:_____

_____

Do you smoke? _____ Yes _____ No

_____ previously smoker: _____ per day _____ stopped date: _____

_____ currently smoking: _____ per day

Do you do any regular exercises? _____ Yes _____ No

If yes, specify:

_____

Do you drink alcohol? _____ Yes _____ No

_____ previously drinker: _____ per day _____ stopped date: _____

_____ currently drinking: _____ per day

DEMOGRAPHIC 12 MONTHS

CODE: _____ **Date:** _____

Surname: _____ **Name:** _____ **Sex:** _____ **Age:** _____

Date of Birth: _____ **Marital Status:** _____ **Telephone No:** _____

Is your address the same as before? _____ Yes _____ No

If No, please specify: _____

Is your family doctor / specialist the same as before? _____ Yes _____ No

If No, please specify: _____

Have you had any health problem in the last 9 months? ___ Yes _____ No

If yes, specify:

_____

Do you take any medication? _____ Yes _____ No

If yes, please specify:

_____

_____

Do you smoke? _____ Yes _____ No

_____ previously smoker: _____ per day _____ stopped date: _____

_____ currently smoking: _____ per day

Do you do any regular exercises? _____ Yes _____ No

If yes, specify: _____

Do you drink alcohol? _____ Yes _____ No

_____ previously drinker: _____ per day _____ stopped date: _____

_____ currently drinking: _____ per day

MEASUREMENTS (0 time, 3 months, 12 months)

CODE: _____ Date: _____

Surname: _____ Name: _____

Weight: _____ Height: _____ BMI: _____

Waist: _____ Hip: _____ W:H ratio: _____ BSA: _____

Body density: _____ Body fat %: _____

Blood Pressure: _____ Pulse: _____

Skinfold thickness:

1 _____ 2 _____ 3 _____triceps _____

1 _____ 2 _____ 3 _____biceps _____

1 _____ 2 _____ 3 _____subscapular _____

1 _____ 2 _____ 3 _____suprailiac_____

Skinfold Sum:

Hypoglycaemic medication intake during the 12 months in Control group (C1-C24):

CODE	ON ADMISSION	3 MONTHS	12 MONTHS
C1			
C2	Gliclazide 80 mg, tab. b.d	Gliclazide 80 mg. b.d	Gliclazide 80 mg tab. b.d
C3	Glibenclamide 5 mg t.d.s, Metformin 500 mg, tab. daily	Glibenclamide 5 mg t.d.s, Metformin 500 mg tab. daily	Glibenclamide 5 mg t.d.s, Metformin 500 mg tab. daily
C4			
C5			
C6			
C7			
C8			
C9			
C10			
C11	Gliclazide 80 mg tab. b.d	Gliclazide 80 mg tab. b.d	Gliclazide 80 mg tab. b.d
C12			
C13	Metformin 500 mg 1 tab. daily	Metformin 500 mg tab. daily	Metformin 500 mg tab. daily
C14			
C15			
C16			
C17	Metformin 500 mg tab. daily	Metformin 500 mg tab. daily	Metformin 500 mg tab. daily
C18	Tolbutamide 1 tab. b.d	withdrew	withdrew
C19			
C20			
C21			
C22			
C23	Metformin 500 mg tab. daily	withdrew	withdrew
C24			

Hypoglycaemic medication intake during the 12 months in HMUFA group (P1-P25):

CODE	ON ADMISSION	3 MONTHS	12 MONTHS
P1			
P2			
P3			
P4			
P5			
P6	Gliclazide 80 mg 1/2 tab. b.d	Gliclazide 80 mg 1/2 tab. b.d	Gliclazide 80 mg 1/2 tab. b.d
P7	Gliclazide 80 mg 1/2 tab. daily	Gliclazide 80 mg 1/2 tab. daily	Gliclazide 80 mg 1/2 tab. daily
P8			
P9			
P10	Gliclazide 80 mg 1/2 tab. daily	Gliclazide 80 mg 1/2 tab. daily	Gliclazide 80 mg 1/2 tab. daily
P11			
P12			
P13	Metformin 500 mg 2 tab. t.d.s	Metformin 500 mg 2 tab. t.d.s	Metformin 500 mg 2 tab. t.d.s
P14			
P15			
P16			
P17			
P18			
P19			
P20			
P21			
P22			
P23			
P24	Metformin 500 mg tab. t.d.s, Gliclazide 80 mg 1 tab. b.d	Metformin 500 mg tab. t.d.s, Gliclazide 80 mg 1 tab. b.d	withdrew
P25			

Hypertensive medication intake during the 12 months in Control group (C1-C24):

CODE	ON ADMISSION	3 MONTHS	12 MONTHS
C1			
C2			
C3	Prazosin 5 mg, tab. b.d, Enalapril 2.5 mg, tab. b.d, Indapamide hemihydrate 2.5 mg, tab. daily	Prazosin 5 mg, tab. b.d, Enalapril 2.5 mg, tab. b.d, Indapamide hemihydrate 2.5 mg, tab. daily	Prazosin 5 mg, tab. b.d, Enalapril 2.5 mg, tab. b.d, Indapamide hemihydrate 2.5 mg, tab. daily
C4	Enalapril.5 mg, tab. daily	Enalapril.5 mg, tab. daily	Enalapril.5 mg, tab. daily
C5			
C6			
C7			
C8			
C9	Atenolol 50 mg, tab. daily	Atenolol 50 mg, tab. daily	Atenolol 50 mg, tab. daily
C10			
C11			
C12			
C13			
C14			
C15			
C16			
C17	Enalapril 10 mg, 1 tab. daily.	Enalapril 10 mg, 1 tab. daily.	Enalapril 10 mg, 1 tab. daily.
C18			
C19	Amlodipine besylate 10 mg, tab. daily.	Amlodipine besylate 10 mg, tab. daily.	Amlodipine besylate 10 mg, tab. daily.
C20			
C21			
C22	Metoprolol tartrate 50 mg, 1 tab. daily, Fosinopril sodium 10 mg, tab. daily.	Metoprolol tartrate 50 mg, 1 tab. daily, Fosinopril sodium 10 mg, tab. daily.	Metoprolol tartrate 50 mg, 1 tab. daily, Fosinopril sodium 10 mg, tab. daily.
C23	Labetalol 100 mg, 1 tab. b.d	withdrew	withdrew
C24			

Hypertensive medication intake during the 12 months in HMUFA group (P1-P25):

CODE	ON ADMISSION	3 MONTHS	12 MONTHS
P1	Captopril 12.5 mg, 2 tab. b.d, Felodipine 5 mg 1 tab. daily.	Captopril 12.5 mg, 2 tab. b.d, Felodipine 5 mg 1 tab. daily.	Captopril 12.5 mg, 2 tab. b.d, Felodipine 5 mg 1 tab. daily.
P2			
P3	Verapamil 240 mg tab. daily., Captopril 25 mg, tab. b.d	Verapamil 240 mg tab. daily., Captopril 25 mg, tab. b.d	Verapamil 240 mg tab. daily., Captopril 25 mg, tab. b.d
P4	Atenolol 50 mg, 1/2 tab. daily	Atenolol 50 mg, 1/2 tab. daily	Atenolol 50 mg, 1/2 tab. daily
P5			
P6	Propranolol 120 mg, tab. daily.	Propranolol 120 mg, tab. daily.	Propranolol 120 mg, tab. daily.
P7			
P8			
P9			
P10			
P11			
P12			
P13			
P14			
P15			
P16			
P17			
P18			
P19			
P20			
P21			
P22			
P23			
P24	Verapamil 375 mg, tab. daily	Verapamil 375 mg, tab. daily	withdrew
P25			

Hypercholesterolemia medication intake during the 12 months in Control group (C1-C24):

CODE	ON ADMISSION	3 MONTHS	12 MONTHS
C1			
C2			
C3			
C4			
C5			
C6			
C7			
C8			
C9	Gemfibrozil 600 mg, tab. b.d	Gemfibrozil 600 mg, tab. b.d	Gemfibrozil 600 mg, tab. b.d
C10			
C11			
C12			
C13			
C14			
C15			
C16			
C17			
C18			
C19			
C20			
C21			
C22			
C23			
C24			

Hypercholesterolemia medication intake during the 12 months in HMUFA group (P1-P25):

CODE	ON ADMISSION	3 MONTHS	12 MONTHS
P1			
P2	Simvastin 5 mg, tab. b.d	Simvastin 5 mg, tab. b.d	medication stopped
P3	Gemfibrozil 600 mg, tab. b.d	Gemfibrozil 600 mg, tab. b.d	Gemfibrozil 600 mg, tab. b.d
P4			
P5			
P6			
P7	Gemfibrozil 600 mg, tab. b.d	Gemfibrozil 600 mg, tab. b.d	Gemfibrozil 600 mg, tab. b.d
P8	Simvastin 10 mg, tab. b.d	Simvastin 10 mg, tab. b.d	medication stopped
P9			
P10			
P11			
P12			
P13			
P14			
P15			
P16			
P17			
P18			
P19			
P20			
P21			
P22			
P23			
P24	Gemfibrozil 600 mg, tab. b.d	Gemfibrozil 600 mg, tab. b.d	withdrew
P25			

Cardiac drugs and other medication intake during the 12 months in Control group (C1-C24):

CODE	ON ADMISSION	3 MONTHS	12 MONTHS
C1			
C2	Thyroxine 0.1 mg, daily	Thyroxine 0.1 mg, daily	Thyroxine 0.1 mg, daily
C3			
C4	Allopurinol 100 mg, daily	Allopurinol 100 mg, daily	
C5			
C6	Allopurinol 300 mg, daily	Allopurinol 300 mg, daily	Allopurinol 300 mg, daily
C7			
C8			
C9			
C10			
C11			
C12			
C13			
C14	Indocid 1 tab. q.i.d, Allopurinol 600 mg, 1 tab. daily	Indocid 1 tab. q.i.d, Allopurinol 600 mg, 1 tab. daily	Indocid 1 tab. q.i.d, Allopurinol 600 mg, 1 tab. daily
C15	Cardizem 60 mg, 1 tab. t.d.s Imdure 60 mg, daily	Cardizem 60 mg, 1 tab. t.d.s Imdure 60 mg, daily	Cardizem 60 mg, 1 tab. t.d.s Imdure 60 mg, daily
C16			
C17		Aspirin tab. p.r.n	
C18		withdrew	withdrew
C19	Zantac 150 mg, 1 tab. b.d	Zantac 150 mg, 1 tab. b.d	Zantac 150 mg, 1 tab. b.d
C20			
C21			
C22			
C23	Thyroxine 1 tab. b.d	withdrew	withdrew
C24			

Cardiac drugs and other medication intake during the 12 months in HMUFA group (P1-P25):

CODE	ON ADMISSION	3 MONTHS	12 MONTHS
P1			
P2	Zantac 150 mg, 1 tab. b.d, Aspirin 1/2 tab. daily	Zantac 150 mg, 1 tab. b.d, Aspirin 1/2 tab. daily	Zantac 150 mg, 1 tab. b.d, Aspirin 1/2 tab. daily
P3			
P4			
P5			
P6			
P7			
P8	Diltiazem 60 mg, tab. daily	Diltiazem 60 mg, tab. daily	Diltiazem 60 mg, tab. daily
P9	Theodur 300 mg, 1 tab. b.d- Ventolin and Becotide puff p.r.n	Theodur 300 mg, 1 tab. b.d- Ventolin and Becotide puff p.r.n	Theodur 300 mg, 1 tab. b.d- Ventolin and Becotide puff p.r.n
P10	Tryptanol 25 mg, 1 Tab. b.d	Tryptanol 25 mg, 1 Tab. b.d	Tryptanol 25 mg, 1 Tab. b.d
P11			
P12	Allopurinol 600 mg tab. p.r.n	Allopurinol 600 mg tab. p.r.n	withdrew
P13			
P14	Ventolin puff p.r.n	Ventolin puff p.r.n	Medication stopped
P15			
P16	Ventolin puff p.r.n	Ventolin puff p.r.n	Medication stopped
P17			
P18			
P19			
P20	Allopurinol 600 mg tab. p.r.n - Aspirin 150 mg, daily	Allopurinol 600 mg tab. p.r.n - Aspirin 150 mg, daily	Allopurinol 600 mg tab. p.r.n - Aspirin 150 mg, daily
P21			
P22			
P23			
P24			
P25			

APPENDIX II

Chemical Solutions and Assays

1.HDL determination in plasma

Procedure:

1. Allow specimens and precipitation reagents to equilibrate to room temperature.
2. Using a manual pipette, transfer 1.0 ml. aliquot of plasma into appropriately labelled eppendrof tubes.
3. Add 100µL of the combined of detrain sulfate-Mg^{2+} solution to each tube. Immediately after addition of this reagent, mix the contents of each tube in sequence for at least 3 seconds, with a vortex-type mixer.
4. Allow the tubes to stand at room temperature for 10 minutes before sedimenting the insoluble lipoproteins by centrifugation.
5. Centrifuge tubes for 30 minutes with a refrigerated centrifuge (4°C, 1500×g) or with an unrefrigerated benchtop centrifuge at 1000 rpm for 15 minutes.
6. Remove tubes from the centrifuge and inspect supranatants for turbidity. Transfer the clear supernatants solution by a Pasture pipette to a second labelled vial for later compositional analysis.

Note: Any turbidity or cloudiness in the supranatant indicates incomplete sedimentation of VLDL, IDL, and LDL and consequent contamination and overestimation of HDL. This is usually observed in high triglycerides levels in samples.

7. Turbid supranatant can be conveniently cleared by one of the following methods:

(a) Without separating the turbid supranatant from the precipitate, add to the separation tube 1.0 ml. of 0.15 mol/L NaCl solution and another 100µL of combined precipitant reagent.

Mix thoroughly with a vortex-type mixer, then centrifuge as previously described.

Obtain the clear supranatant for analysis. Supernatant cholesterol must be multiplied × 2 to correct for this dilution.

(b) Alternatively, the turbidity can be removed by ultrafiltration with an 0.22 µm filter.

2. *Procedure for HDL Radiolabelled in the cholestryl ester moiety*

1. A volume of 50 ml human plasma (from a healthy volunteer) was adjusted to density of 1.13 g/ml with solid KBr and ultracentrifuged for 24 hours at 49,000 rpm in a TL-50.3 rotor at, at 4°C, by using a L8-70 Centrifuge (Beckman, USA).

2. The plasma fraction of d > 1.13 g/ ml was recovered by tube slicing and dialyzed overnight against TBS (buffer was changed several times during dialyzing period).

3. A quantity of 200 µmol of [1α, 2α (n)-^{3}H]-cholesterol (specific activity, 46.3 Ci /mmol) in plastic scintillation vial was evaporated to dryness under a stream of nitrogen for approximately 1 hour.

4. Dried radiolabelled cholesterol was then redissolved in 50 µl of ethanol.

5. The plasma fraction of 1.13 g/ml was added to the radiolabelled cholesterol solution under gentle stirring.

6. The mixture was incubated for 24 hours at 37°C in a shaking water bath to allow cholesterol esterification by the LCAT (lecithin:cholesterol acyltransferase) reaction.

7. After incubation period, the solution was measured and adjusted to density 1.13 g/ml with solid KBr and subjected to centrifugation, for 24 hours at 49000 rpm in a 50.3 rotor, at 4°C, by using a L8-70M centrifuge (Beckmann, USA). The aim of this last centrifugation was to remove any radiolabelled lipoproteins of density < 1.13 g/ml, possibly produced during the incubation period.

8. After slicing, infranatant was collected in cylinder and measured.

9. The measured plasma fraction was adjusted to density >1.21 g/ml with solid KBr and centrifugated for 40 hours at 49000 rpm in a TL-50.3 rotor at 4°C.

10. After slicing, supernatant was recovered as HDL_3 radiolabelled.

11. (Washing Spin): KBr solution density = 1.21 was added to HDL3 radiolabelled sample and subjected to ultracentrifugation for 16 hours at

100000 rpm in a TLA-100.4 rotor, at 4°C, by using a tabletop TL-100 centrifuge (Beckmann, USA)

12. After slicing supranatant was dialyzed against TBS for 24 hours (buffer was changed several times).

13. Thin layer chromatography (TLC) was done on radiolabelled HDL_3. Approximately 95 % of total radioactivity was in cholesteryl ester (CE) as ascertained by (TLC).

3. TLC determination of cholesterol ester (CE) in radiolabelled HDL3:

Prepare following solutions:

1. Extraction solution

160 ml isopropanol
40 ml n-heptane
4 ml 1 N (0.5 M) H_2SO_4

2. Developing solution for TLC plates

45 ml hexane
20 ml diethyl ether
1.5 ml methanol
1 ml acetic acid

The recovery of cholesterol ester is determined by adding a know amount of ^{14}C-cholesteryl oleate to the extraction solution. Prepare stock solution by adding 10 ul ^{14}C- cholesteryl oleate to 100 ml extraction solution. Prepare working solution by diluting stock solution 1:8 with extraction solution (ie. 0.5 ml stock + 3.5 ml extraction solution). From this, take 2.5 ml at next step.

procedure:

1. Add 5 µl radiolabelled HDL_3 or radiolabelled LDL to 495 µl TBS buffer (pH = 7.4).

2. Add 40 µl unlabelled HDL_3 or LDL (carrier lipoprotein)

3. Add 2.5 ml extraction solution (with ^{14}C-cholesteryl oleate)

4. Cap and vortex

5. Stand at room temperature 20 minutes
6. Add 1.5 ml n-heptane and 1 ml water
7. Cap and vortex
8. Stand at room temperature for 15 minutes. Prepare tank and TLC plate
9. Transfer 800 µl of heptane (top) phase to a scintillation vial labelled 'CE' (cholesteryl ester)
10. Transfer 400 µl of heptane (top) phase to a scintillation vial label TC (total count)
11. Dry sample under N_2 gas
12. Add 10 ml scintillation fluid to TC vial
13. Add 200 µl chloroform to CE vial and dissolve dried lipid
14. Spot 100 µl of solution from CE vial onto silica gel TLC plate
15. Put the silica gel TLC plate in tank and develop until solvent is front about 2 cm from top of the silica gel TLC plate (CE runs with solvent front)
16. Remove the silica gel TLC plate from tank and dry in fume cupboard
17. Develop with iodine (few crystals)
18. Scrape CE band into scintillation vial. Add 10 ml scintillation fluid
19. Express CE counts as % of total ^3H counts
20. Calculate recovery of 14C-cholesteryl oleate

    CE vial count / TC vial count × 100 = X (% recovery of CE)

4. CETP activity % in plasma Procedure:

1. 100 µl plasma was mixed with 50 µl [^3H] HDL3 (containing 2.5 nmol cholesterol. 2.2×10^5 counts/minute per 50 µl)
2. Incubate it for 3 hours at 37°C.
3. Place the tubes immediately on ice.

4. Precipitated the apo B containing lipoproteins with dextran sulfate-Mg^{2+} (Warnick et al., 1982)

5. Supranatants (0.8 of total volume) were collected in plastic scintillation vials. A volume of 10 mL of scintillation fluid (Instagel, Pachard Instrument Company, IL, USA) was added to each vial and the radioactivity was assayed for 10 minute in a 'Hewlett-Packard scintillation counter'.

The percentage of radioactivity transferred to apo B containing lipoproteins was calculated, as the difference between the total radioactivity of the assay and the radioactivity of the supranatant after precipitation of the VLDL, IDL, and LDL, with adjustment for non-specific transfer.

The CETP assay procedure is summarized in Table 1

Table-1: Summerized CETP assay procedure

Tube	TBS	HDL_3	plasma	Total volume	Incub Period	15 µl	Total volume	Recover volume
1,2	100 µl	50 µl	-	150 µl	3.h	TBS	165 µl	132 µl
3,4	100 µl	50 µl	-	150 µl	3.h	Dextr sulfate	165 µl	132 µl
5,6	-	50 µl	100 µl (+cont)	150 µl	3.h	Dextr sulfate	165 µl	132 µl
7,8	-	50 µl	subject sample	150 µl	3.h	Dextr sulfate	165 µl	132 µl
9,10	-	50 µl	subject sample	150 µl	3.h	Dextr sulfate	165 µl	132 µl
.....	.....	.....	.....	....	.....		.....	.....

Incub = Incubation, h = hours, Dextr = Dextran, (+ cont) = positive control, Recover= Recovery.

UNIMATE 3 APOA Art. 07 3688 0

Apolipoprotein A-1

4 x 10.5 mL

Intended use
Unimate 3 APOA is an in vitro diagnostic reagent intended for the quantitative immunological determination of human apolipoprotein A-1 (Apo A-1) in serum.

Method
Immunoturbidimetric.

Principle
Human apolipoprotein A-1 forms a precipitate with a specific antiserum which is determined turbidimetrically (measurement of cloudiness) at 340 nm (fixed time method).

Specimen
Serum.
Samples should be fresh, stored for a maximum of 1 week at +2 to +8 °C or stored frozen once at –20 °C.

Reference values

Apolipoprotein A-1	
Females	115–220 mg/dL (1.15–2.2 g/L)
Males	115–190 mg/dL (1.15–1.9 g/L)
Apolipoprotein B	
Females	60–150 mg/dL (0.6–1.5 g/L)
Males	70–160 mg/dL (0.7–1.6 g/L)
Ratio Apolipoprotein B/Apolipoprotein A-1	
Females	0.35–1.15
Males	0.45–1.25

For the IFCC Reference Preparation SP1 (October 1992 WHO-IRP) for apolipoprotein A-1 and the IFCC Reference Preparation SP3-07 for apolipoprotein B the following additional reference values apply:

	Apolipoprotein A-1	Apolipoprotein B
Females	1.20–2.20 g/L	0.55–1.25 g/L
Males	1.10–2.00 g/L	0.55–1.35 g/L

Quality control
Apolipoproteins T Control, art. 07 3721 6 (US # 42391)

Kit contents
R Reagent, 4 x 10.5 mL
Anti-Apo A-1 T antiserum (sheep)
Antiserum specific for human apolipoprotein A-1, in phosphate buffer stabilized with 0.09% sodium azide.

Reagents
Working reagent
The reagent R is ready to use. Warm up the reagent to room temperature prior to assay.
The reagent, once opened is stable for 4 weeks at +2 to +8 °C in the original bottle if it is immediately and tightly closed after use.

Additionally required (not delivered with the kit):
Apolipoproteins T Standard, art. 07 3720 8 (US # 42390)
Apolipoproteins T Control, art. 07 3721 6 (US # 42391)
NaCl solution 154 mmol/L (0.9%)

Procedure
For applications on COBAS® instruments please refer to the corresponding test instruction manuals.

Notes
Measuring range: 35–550 mg/dL (0.35–5.5 g/L).
The measuring range is determined by the batch specific apolipoprotein A-1 in the standard serum and the serial dilutions selected.
The reagent R contains < 0.1% sodium azide as preservative. Avoid swallowing and contact with skin or mucous membranes.

References

1. Becker, W., Rapp, W., Schenk, H.G., Störiko, K.: *Z Klin Chem Klin Biochem 6,* 113–122 (1968).
2. Thomas, L.: *Labor und Diagnose,* 1. Ed., p. 628. Marburg/Lahn: Die Medizinische Verlagsgesellschaft, 1978.
3. Dati, F., Lammers, M., Adam, A., Sondag, D., Stienen, L.: *Lab Med 13,* 87–90 (1989).
4. Albers, J.J., Marcovina, S.M., Kennedy, H.: *Clin Chem 38,* 658–662 (1992).
5. Dati, F.: IFCC program for the standardization of assays for apolipoprotein [illegible] *in Clinical Biochemistry,* pp. 419–423. Ed. K. Miyai, [illegible]a. New York: Elsevier, 1992.

UNI-KIT

Apolipoprotein B T Antiserum

Roche

1337289
P-2995/C
e d f i esp
Ed. Nov. 90

Art. 07 2995 7 (4 x 10.5 ml)

Antiserum (rabbit) for the quantitative immunological determination of human apolipoprotein B in serum.

For in vitro diagnostic use

Also required:
T Standard Apolipoproteins (Roche) (art. 07 3068 8)
NaCl solution 154 mmol/l (0.9%)

General information

Method
Immunoturbidimetric.

Principle[1]
Human apolipoprotein B forms with a specific antiserum a precipitin which is determined turbidimetrically (measurement of cloudiness) by a fixed time method at 340 nm (or 334 or 365 nm).

Sample[2]
Fresh serum or stored for a maximum of 1 week at +2 to +8 °C. Very turbid (lipemic) samples should be diluted prior assay.

Package
T Antiserum Apolipoprotein B 10.5 ml per bottle
Antiserum specific for human apolipoprotein B in phosphate buffer stabilized with sodium azide.
The bottles, when unopened, are stable at +2 to +8 °C until the expiry date stated on the labels.

Reference values[3]

Apo A-1		
Male	1.15–1.90 g/l	(median 1.45 g/l)
Female	1.15–2.20 g/l	(median 1.60 g/l)
Apo B		
Male	0.70–1.60 g/l	(median 1.15 g/l)
Female	0.60–1.50 g/l	(median 1.05 g/l)
Ratio Apo B/Apo A-1		
Male	0.45–1.25	(median 0.80)
Female	0.35–1.15	(median 0.65)

Quality control
T Control Apolipoproteins (Roche) (art. 07 3069 6)

Manual procedure

Preparations

Reagent
The T Antiserum Apolipoprotein B is ready to use. Prior assay warm up the reagent to room temperature. The reagent, once opened is stable 4 weeks at +2 to +8 °C, 1 week at +15 to +25 °C in the original bottle if it is immediately and tightly closed after use.

Samples and controls
Dilute the samples and controls with NaCl (0.9%) in ratio 1:21 (1+20).

Main standard
Reconstitute T Standard Apolipoproteins (Roche) with 0.5 ml of distilled water. Allow to stand at +15 to +25 °C for 30 minutes. Then gently swirl for 1 minute. The reconstituted standard is ready to use and is stable for 1 week at +2 to +8 °C.

Standard serial dilutions
Dilute the reconstituted T Standard Apolipoproteins (Roche) with NaCl solution in a geometric series, starting with 1:6 in order to have the following series: 1:6, 1:12, 1:24, 1:48, 1:96. For diluting this pipetting schema can be used:

Solutions	Dilutions 1:6	1:12	1:24	1:48	1:96
T Standard reconstituted	100				
Dilution 1:6		300			
Dilution 1:12			300		
Dilution 1:24				300	
Dilution 1:48					300
NaCl 0.9%	500	300	300	300	300

The dilutions of the standard are stable during 1 week at +2 to +8 °C if stored in closed vials (risk of contamination).

Bibliographie

1. Becker, W., Rapp, W., Schenk, H. G., Störiko, K.: *Z Klin Chem Klin Biochem 6*, 113–122 (1968).
2. Thomas, L.: *Labor und Diagnose*, 1. Ed., p. 628. Marburg/Lahn: Die Medizinische Verlagsgesellschaft, 1978.
3. Dati, F., Lammers, M., Adam, A., Sondag, D., Stienen, L.: *Lab Med 13*, 87–90 (1989).

CHOL

MPR 1	1 442 341	10 x 32 ml	1
MPR 2	1 442 350	10 x 100 ml	2
MPR 3	236 691	4 x 500 ml	2

Cholesterol

CHOD-PAP method

Method

Siedel, J., et al. (1983). *Clin. Chem.* 29:1075.
Kattermann, R., et al. (1984). *Clin. Chem. Clin. Biochem.* 22: 245.
Trinder, P. (1969). *Ann. clin. Biochem.* 6:24.

Test principle

cholesterol esters + H_2O $\xrightarrow{\text{cholesterol esterase}}$ cholesterol + RCOOH

cholesterol + O_2 $\xrightarrow{\text{cholesterol oxidase}}$ Δ^4-cholestenone + H_2O_2

2 H_2O_2 + 4-aminophenazone + phenol $\xrightarrow{\text{POD}}$ 4-(*p*-benzoquinone-monoimino)-phenazone + 4 H_2O

Clinical interpretation according to the recommendations of the European Atherosclerosis Society[1]:

		Lipid disorder
Cholesterol Triglycerides	< 200 mg/dl < 200 mg/dl	No
Cholesterol	200–300 mg/dl	Yes, if HDL cholesterol < 35 mg/dl
Cholesterol Triglycerides	> 300 mg/dl > 200 mg/dl	Yes

Values in mmol/l:

Cholesterol: 35 mg/dl = 0.9 mmol/l
200 mg/dl = 5.2 mmol/l
300 mg/dl = 7.8 mmol/l

Triglycerides: 200 mg/dl = 2.3 mmol/l

Sample material

Serum, heparinized plasma or EDTA plasma
Stable for six days at +4 to 25°C
four months at –20°C.

Contents	MPR 1	MPR 2	MPR 3	Preparation and stability of the reagent solution
Bottle 1 Cholesterol reagent	10	10	4	MPR 1: Dissolve contents of one bottle 1 by adding 32 ml redist. water. MPR 2: Dissolve contents of one bottle 1 by adding 100 ml redist. water. MPR 3: Dissolve contents of one bottle 1 by adding 500 ml redist. water. *The reagent solution is ready to use after 10 minutes.* Stable for four weeks at +2 to 8°C seven days at +15 to 25°C.

Procedure

Wavelength: Hg 546 nm (470–560 nm)
Spectrophotometer: 500 nm
Cuvette: 1 cm light path
Incubation temperature: 20–25°C or 37°C
Measure against reagent blank (RB).
One reagent blank is sufficient for each assay series.

Pipette into test tubes:		
	RB	Sample
sample material	–	0.02 ml
reagent solution	2.00 ml	2.00 ml
Mix, and incubate RB and sample for 10 min at 20–25°C or 5 min at 37°C. Read absorbance of sample against RB within 1 hour = A_{sample}.		

Dilution threshold

1000 mg/dl (25.9 mmol/l)
At higher cholesterol concentrations, dilute 0.1 ml of sample material with 0.2 ml of 0.9% NaCl solution and repeat assay (result x 3).

Calculation

of the concentration (c) of cholesterol in the sample:

Wavelength	mg/dl	mmol/l
Hg 546 nm	$c = 853 \times A_{sample}$	$c = 22.1 \times A_{sample}$
500 nm	$c = 575 \times A_{sample}$	$c = 14.9 \times A_{sample}$

Concentrations of the reagent solution

Tris buffer: 100 mmol/l, pH 7.7; Mg^{2+}: 50 mmol/l; 4-aminophenazone: 1 mmol/l; sodium cholate: 10 mmol/l; phenol: 6 mmol/l; 3,4-dichlorophenol: 4 mmol/l; fatty alcohol polyglycol ether: 0.3%; cholesterol esterase ≧ 0.4 U/ml; cholesterol oxidase ≧ 0.25 U/ml; peroxidase ≧ 0.2 U/ml

Quality control

Accuracy: Precinorm® U, Precinorm® L, Precipath® U, Precipath® L
Precision: Precinorm® UPX

Please note

If difficulties encountered in quality control point to inadequate linear response on the part of the photometer, or if measurements cannot be taken at Hg 546 nm or 500 nm, a calibration curve must be constructed using Preciset® Cholesterol (Cat.No. 125 512). The cholesterol values are read off the curve.

Bilirubin concentrations above 4 mg/dl interfere with the test (results up to 10% too low).

Hemoglobin concentrations up to 200 mg/dl do not affect the test.

Reference

1 Study Group, European Atherosclerosis Society. Strategies for the prevention of coronary heart disease: A policy statement of the European Atherosclerosis Society. (1987). *Eur. Heart J.* 8:77.

April 1993

493.1264.0381 101 (i)

BOEHRINGER MANNHEIM

Triglycerides GPO-PAP

High performance
Enzymatic colorimetric test

Peridochrom® Triglycerides GPO-PAP
Cat.No. 701 882 for 6 x 13 ml reagent
Cat.No. 701 904 for 15 x 32 ml reagent

Test-Combination Triglycerides GPO-PAP
Cat.No. 701 912 for 5 x 100 ml reagent

Additional reagent for measurement against standard:
Precimat® Glycerol, Cat.No. 166 588

Method

Enzymatic hydrolysis of triglycerides with subsequent determination of the liberated glycerol by colorimetry.

References:
Mod. according to Wahlefeld, A.W. (1974).
Triglycerides. Determination after Enzymatic Hydrolysis. Page 1831ff. In H.U. Bergmeyer, ed. *Methods of Enzymatic Analysis*, 2nd English ed. (Transl. from 3rd German ed.). Verlag Chemie Weinheim *and* Academic Press, Inc., New York and London, 4 vols.
Nägele, U. (Boehringer Mannheim GmbH), personal communication.
Trinder, P. (1969). *Ann. Clin. Biochem.* 6:24.

Test principle

triglycerides + 3 H_2O —(lipase)→ glycerol + 3 RCOOH

glycerol + ATP —(GK)→ glycerol-3-phosphate + ADP

glycerol-3-phosphate + O_2 —(GPO)→ dihydroxyacetone phosphate + H_2O_2

H_2O_2 + 4-aminophenazone + 4-chlorophenol —(peroxidase)→ 4-(*p*-benzoquinone-mono-imino)-phenazone + 2 H_2O + HCl

Sample material

Serum, heparinized plasma or EDTA plasma

Reagents

1 Buffer solution
2 Reagent strips or lyophilisate

Reagent concentrations in solution

1 Tris buffer: 0.15 mol/l, pH 7.6; magnesium sulphate: 17.5 mmol/l; EDTA, disodium salt: 10 mmol/l; 4-chlorophenol: 3.5 mmol/l; sodium cholate: 0.15%; potassium hexacyanoferrate (II): 6 µmol/l; hydroxypolyethoxy-*n*-alkanes: 0.12%

2 ATP ≥ 0.5 mmol/l; 4-aminophenazone: 0.35 mmol/l; lipase ≥ 3 U/ml; glycerol phosphate oxidase ≥ 2.5 U/ml; glycerol kinase ≥ 0.2 U/ml; peroxidase ≥ 0.15 U/ml

Quality control

For control of accuracy: Precinorm® U, Precinorm® L, Precipath® U, Precipath® L
For precision control: Precinorm® UPX

Preparation and stability of reagent solution

Peridochrom® Triglycerides GPO-PAP
Do not touch the reagent patches or surrounding area.

Immerse one reagent strip in one bottle of buffer solution and use to stir the bottle contents for ca. 10 sec. Allow to stand in buffer solution for 5 min, stir once again for ca. 10 sec, and then discard reagent strip.

Test-Combination Triglycerides GPO-PAP

Connect one bottle 1 to one bottle 2 with one of the adapters provided in the kit and flush several times to ensure complete dissolution of the lyophilisate.

Reagent solution from different bottles may be pooled for large series.

Stable for two weeks at +2 to 8°C
two days at +15 to 25°C.

Sample preparation

The sample can be stored up to three days at +4°C
four months at −20°C.

Procedure

Wavelength: Hg 546 nm
Spectrophotometer: 500 nm
Cuvette: 1 cm light path
Incubation temperature: 20–25°C or 37°C

Measure against reagent blank. One reagent blank is sufficient for each series.

Pipette into test tubes:	
serum or plasma	0.02 ml
reagent solution	2.00 ml
Mix, and incubate at 20–25°C for 10 min. Read absorbance of sample against reagent blank within 60 min = A_{sample}.	

If incubating at 37°C (10 min), the absorbance of the standard must be determined once for each assay series using Precimat® Glycerol instead of serum or plasma. The absorbance of the sample and the standard must be read within 30 min.

Calculation via factor

Obtain the concentration (c) of triglycerides in the sample from the enclosed table of values or calculate as follows:

Incubation temperature: 20–25°C		
Wavelength	c [mg/100 ml]	c [mmol/l]
Hg 546 nm	1040 x A_{sample}	11.9 x A_{sample}
500 nm	760 x A_{sample}	8.66 x A_{sample}

Calculation via standard

Calculate the concentration (c) of triglycerides as follows:

Incubation temperature: 20–25°C or 37°C	
c [mg/100 ml] = 200 x $\frac{A_{sample}}{A_{standard}}$	c [mmol/l] = 2.29 x $\frac{A_{sample}}{A_{standard}}$

If the triglyceride concentration exceeds 1000 mg/100 ml (11.4 mmol/l), dilute sample 1 + 5 with 0.9% NaCl solution and repeat assay (result x 6).

Clinical interpretation

according to the recommendations of the European Atherosclerosis Society:

		Lipid disorder
Cholesterol Triglycerides	< 200 mg/100 ml < 200 mg/100 ml	No
Cholesterol	200–300 mg/100 ml	Yes, if HDL cholesterol < 35 mg/100 ml
Cholesterol Triglycerides	> 300 mg/100 ml > 200 mg/100 ml	Yes

Values in mmol/l:

Cholesterol: 35 mg/100 ml = 0.9 mmol/l
200 mg/100 ml = 5.2 mmol/l
300 mg/100 ml = 7.8 mmol/l

Triglycerides: 200 mg/100 ml = 2.3 mmol/l

Reference: Study Group, European Atherosclerosis Society.
Strategies for the prevention of coronary heart disease:
A policy statement of the European Atherosclerosis Society.
(1987).
Eur. Heart J. 8:77.

Boehringer Mannheim GmbH
Diagnostica

March 1991

Test-Combination

Free Cholesterol

CHOD-PAP method

Enzymatic colorimetric method

Cat. No. 310 328 for 2 x 90 ml

Method

Stähler, F., et al. (1977). *Med. Lab.* 30: 29.
Trinder, P. (1969). *Ann. clin. Biochem.* 6: 24.

Test principle

Cholesterol + O_2 $\xrightarrow{\text{cholesterol oxidase}}$ Δ^4-cholestenone + H_2O_2

$2\ H_2O_2$ + 4-aminophenazone + phenol $\xrightarrow{\text{POD}}$ 4-(*p*-benzoquinone-monoimino)phenazone + $4\ H_2O$

Normal values for free cholesterol:

32–30% of total cholesterol

Reference: Wybenga, D. R., and J. A. Inkpen. (1974). Lipids. Page 1421 ff *in* Henry, R. J., et al., eds. *Clinical Chemistry: Principles and Technics*, 2nd ed. Harper & Row, Publishers, Inc., New York, San Francisco, and London.

Sample material

Serum, heparinized plasma or EDTA plasma

Reagents

	Contents	Initial concentrations of solutions:
1	Buffer (potassium phosphate)	0.4 mol/l; pH 7.7
	Phenol	20 mmol/l
	methanol	1.85 mol/l
2	Buffer (potassium phosphate)	0.4 mol/l; pH 7.7
	4-Aminophenazone	2 mmol/l
	methanol	1.85 mol/l
	hydroxypolyethoxydodecane	0.4%
3	Cholesterol oxidase	≧ 12 U/ml
	Peroxidase	≧ 8 U/ml

Quality control

For control of accuracy: Precilip® E.L., Precinorm® L

Preparation and stability of solutions

1, 2 and 3 Use solutions undiluted.

Stable up to the expiry date specified when stored at + 2 to + 8°C.

4 Cholesterol reagent:

Pipette 0.5 ml of sol. 3 into bottle 2 and add the entire contents of one bottle 1, or make up stock solution according to the following table:

Number of samples	solution 1, ml	solution 2, ml	solution 3, ml
ca. 4	5	5	0.05
ca. 9	10	10	0.10
ca. 19	20	20	0.20
ca. 39	40	40	0.40

Stable for two weeks at + 2 to + 8°C
two days at + 15 to 25°C.

5 Reagent for sample blank:
(req'd only for assays on hemolytic or turbid sera)

Mix equal volumes of solutions 1 and 2 and store away from light
Stable for two weeks at + 2 to + 8°C
two days at + 15 to 25°C.

Procedure A

Sample material: clear, non-hemolytic serum
Wavelength: Hg 546 nm (470–560 nm)
Spectrophotometer: 500 nm
Glass cuvette: 1 cm light path
Incubation temperature: 20–25°C or 37°C

Measure against reagent blank (RB)

One reagent blank is sufficient for each assay series.

Pipette into cuvette:		
	RB	Sample
sample material	–	0.02 ml
solution 4	2.00 ml	2.00 ml

Mix and incubate RB and sample for 20 min at 20–25°C or 10 min at 37°C. Read absorbance of sample against RB within 2 hours (ΔA).

Procedure B

Sample material: serum or plasma
Wavelength: Hg 546 nm (470–560 nm)
Spectrophotometer: 500 nm
Cuvette: 1 cm light path
Incubation temperature: 20–25°C or 37°C

Measure against sample blank (SB)

Determine ΔA_{RB} once per series to correct for the intrinsic colour of the cholesterol reagent by using redist. water instead of sample in the assay.

Pipette into cuvette:		
	SB	Sample
solution 4	–	2.00 ml
solution 5	2.00 ml	–
sample	0.02 ml	0.02 ml

Mix and incubate SB and sample for 20 min at 20–25°C or for 10 min at 37°C. Read absorbance of sample against SB within 2 hrs., and subtract ΔA_{RB} from ΔA_{sample}. $\Delta A = \Delta A_{sample} - \Delta A_{RB}$

Calculation

of the concentration (c) of free cholesterol in the sample:

Wavelength	mg/100 ml	mmol/l
Hg 546 nm	c = 855 x ΔA	c = 22.1 x ΔA
500 nm	c = 585 x ΔA	c = 15.1 x ΔA

Please note

If difficulties encountered in quality control point to inadequate linear response on the part of the photometer, or if measurements cannot be taken at Hg 546 nm or 500 nm, a calibration curve must be constructed using Preciset® Cholesterol (Cat.No. 125 512). The cholesterol values are read off the curve.

A slight yellow coloration of solution 2 does not affect the test.

Even low concentrations of ascorbic acid and α-methyldopa in the specimen depress the free cholesterol level.

Solutions 1 and 2 contain methanol, which is poisonous. Do not swallow or inhale vapors. Avoid contact with skin. If solution comes into contact with skin or mucous membranes, flush immediately with large quantities of water. If solution comes into contact with eyes, immediately flush liberally with water and consult an ophthalmologist.

Boehringer Mannheim GmbH
Diagnostica

APPENDIX III

DIET

DIETETIC CONSULTATION 1

DATE: **CODE:**

SUBJECT: _____

SEX: _____

AGE: _____

HAVE YOU SEEN A DIETITIAN IN THE PAST?

_____

FOR HOW LONG HAVE YOU BEEN DIAGNOSED WITH NIDDM?

_____

BSL RANGES: _____

HEIGHT: _____(cm) BMI: _____(Kg/m^2)

WEIGHT: _____(Kg)

WEIGHT HISTORY: _____

_____

PAST MEDICAL HISTORY: _____

_____

_____

MEDICATIONS: _____

_____

_____

EMPLOYMENT STATUS: _____

MARITAL STATUS: _____

COUNTRY OF BIRTH: _____

WHO PREPARES THE FOOD AT HOME?

_____

HOW MANY PEOPLE ARE CATERED FOR IN FOOD PREPARATION?

_____

DO YOU UNDERTAKE ANY FORM OF PHYSICAL EXERCISE? YES / NO

* **TYPE OF EXERCISE:** _____

* **DURATION OF EXERCISE:** _____

* **FREQUENCY OF EXERCISE:** _____

DIET HISTORY:

BREAKFAST:

MORNING TEA:

LUNCH:

AFTERNOON TEA:

TEA / DINNER:

SUPPER:

CHECK IF THE FOLLOWING AREAS HAVE BEEN COVERED:

- amount and type of dietary fat
- pasta/rice/starchy vegetables/bread servings/day
- fruit/vegetable serves per day
- serves of meat/poultry/fish/legumes per day
- type and nº of serves milk products per day
- beverages (types and frequency)
- cakes/sweets/ pastries/ biscuits
- salt usage
- food preparation techniques
- portion sizes (esp meat)
- weekday vs weekend consumption patterns
- condiments/sauces/dressings added to food
- takeaway foods / eating out

FREQUENCY OF:

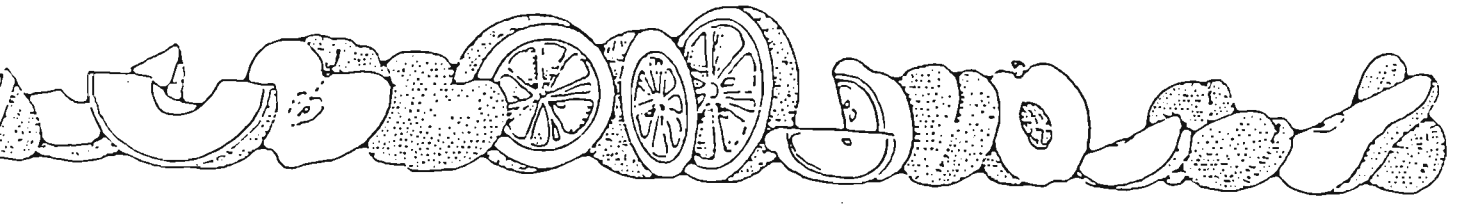
*** avocados:** _____

*** peanut butter:** _____

*** olives:** _____

*** nuts (specify type):** _____

" DIETARY GUIDELINES FOR INDIVIDUALS WITH NON INSULIN DIABETES MELITUS ON A HIGH CARBOHYDRATE DIET"



A HEALTHY DIET AND DIABETES

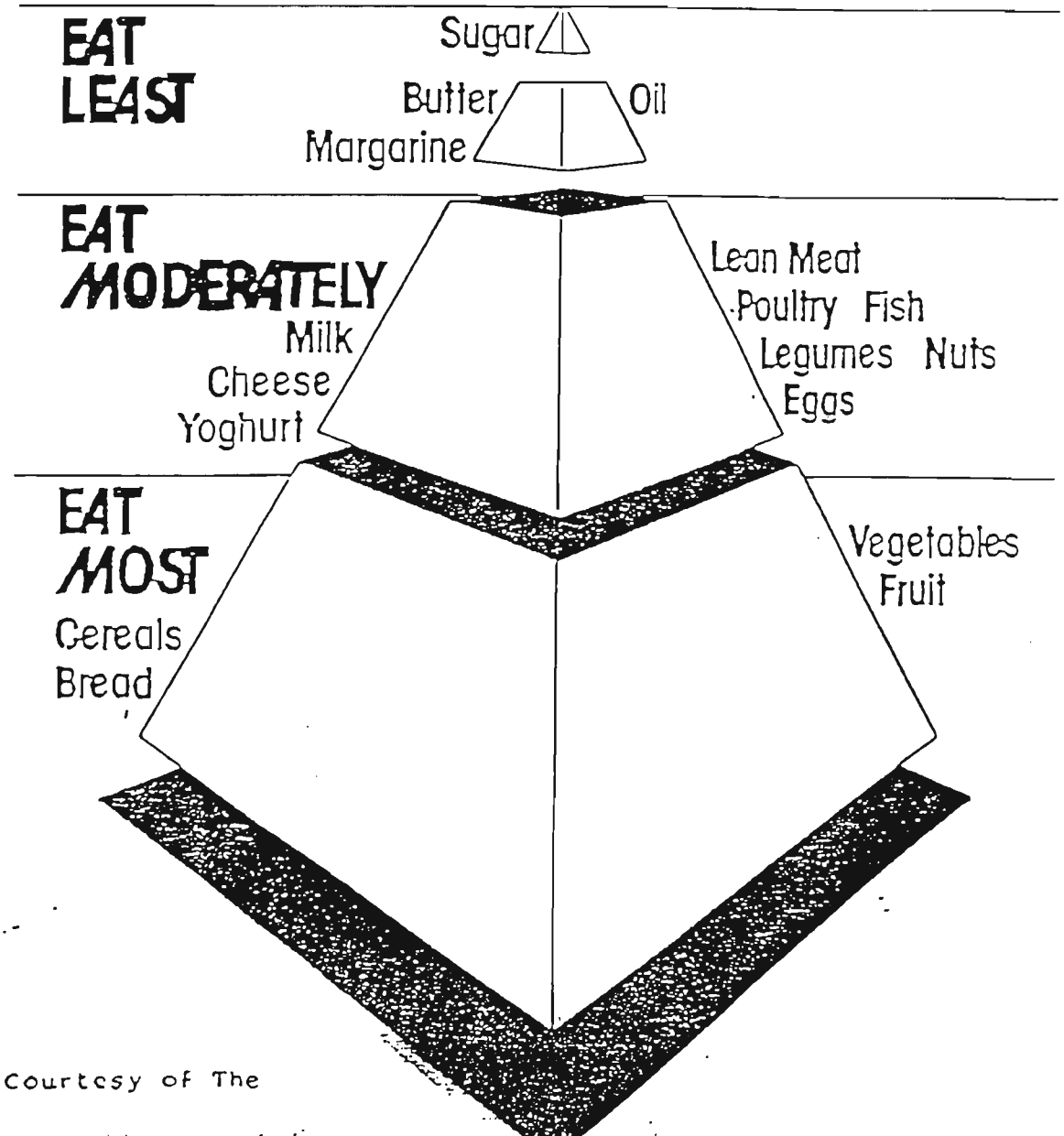
Having diabetes gives you the opportunity to improve your eating habits with immediate and significant benefits.

Healthy eating for diabetes is healthy eating for everyone.

The benefits include:

* Maintenance or achievement of a healthy body weight
* Good control of you blood glucose levels
* Less risk of developing complications of diabetes

Following the *HEALTHY DIET PYRAMID* ensures that your nutritional needs are met.



Courtesy of The

WHAT'S IN FOOD?

Food provides us with different NUTRIENTS which are important for good health. These include protein, fat, carbohydrate, vitamins, minerals, fibre and water.

PROTEIN

* Found in animal foods such as meat, fish, eggs, cheese and vegetable foods like nuts, dried peas and dried beans.
* Your body requires small quantities of protein for body growth and repair.
* EAT SMALL TO MODERATE AMOUNTS

FAT

* Foods high in fat include butter, margarine, oils, cream, chicken skin, fried foods and many take-aways.
* Too much fat can easily make you overweight and increase blood fats.
* EAT LESS FAT

CARBOHYDRATE

* Found in two forms SUGARS AND STARCHES.
* Sugars are added to foods like softdrinks, lollies and cakes.
* Sugars are naturally found in fruit and milk. These foods provide valuable nutrients.
* Starches are found in potatoes, pasta, rice, bread and cereals.
* Carbohydrates are an important fuel for your body.
* Eat foods containing starch at each meal.
* Fruit and milk can be included at each meal.

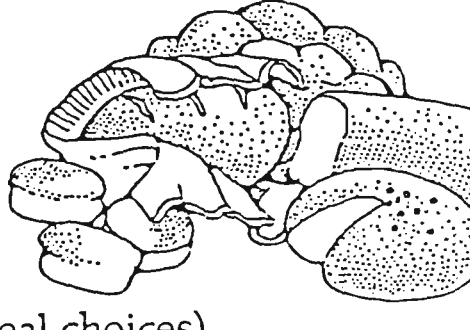
BREADS, CEREALS & GRAINS:

* They are all low in fat, and supply dietary fibre, energy, B vitamins and minerals.

* Try to include at least one serve at each meal from the following list of recommended choices.

* *Recommended choices:*

- bread and bread rolls (especially wholemeal varieties)
- pasta (especially wholemeal)
- rice (especially brown)
- crispbreads (Ryvita/ Vitawheat)
- wholemeal English muffins and crumpets
- Wholegrain cereals (refer to list of best cereal choices)



FRUIT, VEGETABLES & LEGUMES:

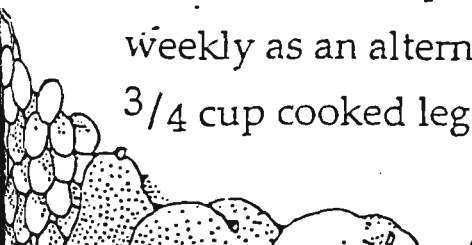
* Fruit, vegetables and legumes are high in fibre and low in fat.

* Include a variety of fruit daily (raw or cooked)

* Include plenty of vegetables daily (especially green and root vegetables)

* Legumes include beans (e.g. baked, kidney, soya, lima and haricot) lentils, split and chick peas. Try to include to include these weekly as an alternative to meat or in meat dishes.

3/4 cup cooked legumes = 75 grams cooked lean meat

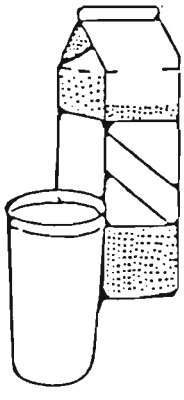


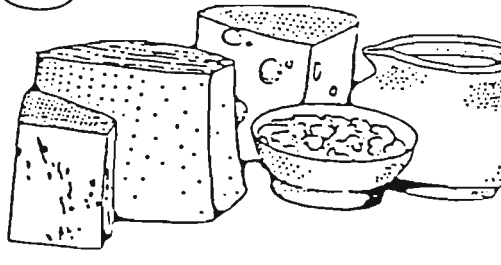
DAIRY PRODUCTS:

* Dairy products provide calcium, protein, vitamins and energy.

* Include low fat and reduced fat dairy products daily to ensure adequate calcium intake.

Recommended choices:



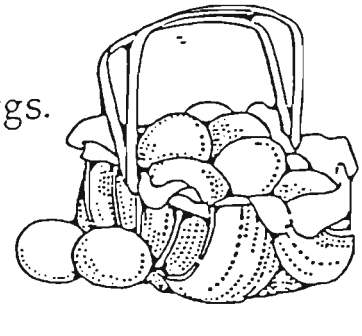


- Farmers Best milk (is reduced fat and contains monounsaturated fat)
- Shape milk (high calcium and low fat)
- Lite White (reduced fat)
- Hi Lite (reduced fat and high calcium)
- Hi Lo (reduced fat)
- Skim milk (low fat)
- reduced fat UHT milk (various brands)
- Diet yoghurt (low fat, artificially sweetened)
- Reduced and low fat cheeses (eg: Devondale 7, cottage, ricotta, Bega super slims, Bega Light ..... plus many more reduced fat varieties).

EGGS:

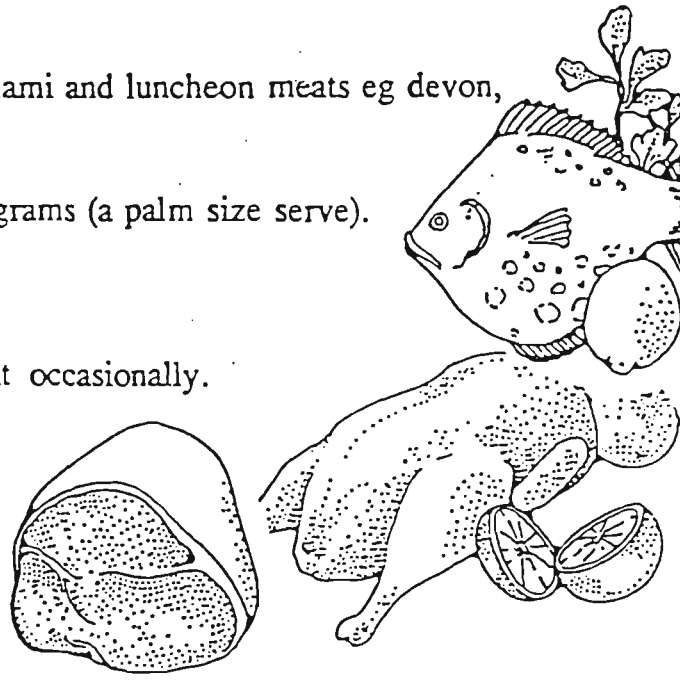
* Maximum of two whole eggs per week (best cooking methods are poaching, boiling, scrambling, microwaving).

* Egg whites may be used instead of whole eggs.



MEATS

* Meat is a good source of protein, energy, vitamins and iron.
* Always trim fat off meat **before** cooking and remove the skin from chicken.
* Grill or roast on a rack so the fat can drip away.
* Cook lean cuts of meat.
* Cut down on sausages, rissoles, bacon, salami and luncheon meats eg devon, chicken loaf.
* Keep the serves small.....about 90 - 100 grams (a palm size serve).
* Include fish at least once or twice a week.
* Substitute 2 eggs for a serving of meat occasionally.



FATS AND OILS:

They must be used in limited amounts as they are high in kilojoules.

HOW TO EAT LESS FAT

When preparing foods:

* Use the very minimum of oil in cooking or none at all.
* Invest in a non-stick frypan and brush with a little oil - do not pour oil in. Alternatively use a cooking spray or pan liners.
* Spread margarine very thinly, or leave it off.
* Use "low-oil" or "no-oil" salad dressings and mayonnaise or use vinegar, lemon juice, garlic and herbs to make your own.
* When you mash potatoes use low-fat milk rather than margarine.
* Serve larger portions of low-fat starchy foods such as pasta, rice, potatoes, vegetables and dried beans.

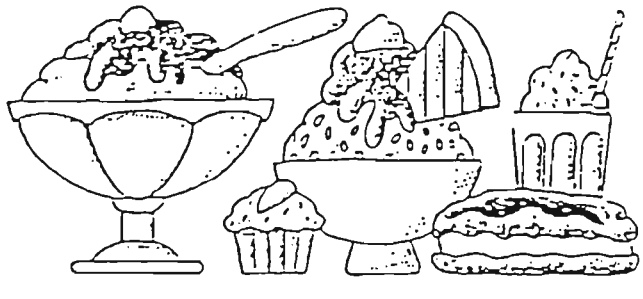
FOODS TO AVOID

Some foods and drinks are discouraged because of their unfavourable effects on blood sugar and blood fat.

It is useful to check food labels or ask your dietitian if you are unsure.

FOODS HIGH IN SUGAR

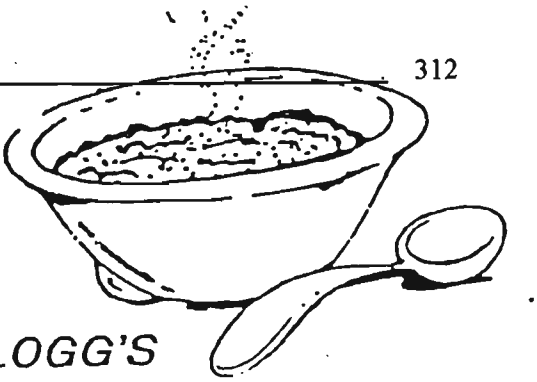
ADDED SUGAR: in tea, coffee, on cereals
SWEET SPREADS: honey, marmalade, syrup, jam
SWEETENED DRINKS: softdrinks, cordial, flavoured mineral water, fruit juice drinks, flavoured milk, fruit nectar, milk shakes
LOLLIES: boiled sweets, mints, toffees, cough lollies, chewing gum, chocolate bars, muesli bars, health bars, diabetic chocolate, glace fruit
SWEET BISCUITS: cream biscuits, chocolate biscuits, shortbread, etc
CAKES: cream cakes, pastries, ice buns, doughnuts
DESSERTS: fruit pies, jelly, sweetened yoghurt, toppings, puddings, fruit in syrup, ice blocks
SWEET CEREALS: Coco-Pops, Nutri-Grain, Honey Smacks, Frosties, Fruit Loops



FOODS HIGH IN FAT

Fatty meat	Frankfurts	Snack foods eg crisps
Salami	Pizza	Pies
Devon	Fried foods	Sausage rolls
Sausages	Pastries	Nuts

BEST CEREAL CHOICES



These are all low in fat, low in sugar and are good sources of fibre.

SANITARIUM

Weet-Bix	)
Bran Bix	) 2
Lite-Bix	) biscuits
Weet-Bix Hi-Bran	)
Weet-Bix plus Oat Bran	1
Good Start	1
Natural Muesli	1/4 cup
Light 'n Tasty	3/4 cup
Puffed Wheat	1 cup

PURINA

Swiss Formula Muesli	1/4 cup
Fruit 'n' Bran Flakes	1/2 cup

FARMLAND

High Fibre	
Breakfast Bran	1/3 cup
Rolled Oats	1/4 cup (raw)
Minute Oats	1/4 cup (raw)

WILLOW VALLEY

Multi Bran	1/3 cup
Crunchy Toasted	
Oat Bran	1/4 cup
Barley = O - S	2/3 cup

CEROLA

Oat Bran Muesli	1/4 cup
Natural Muesli	1/4 cup

KELLOGG'S

All Bran	1/4 cup
Bran Flakes	1/2 cup
Ready-Wheats	13 biscuits
Sultana Bran	1/2 cup
Sustain	1/3 cup
Puffed Wheat	3/4 cup
Balance Oat	
Bran Flakes	2/3 cup
Mini - Wheats	12 biscuits
Mini-Wheats apricot	
or blackcurrant	10 biscuits

UNCLE TOBY'S

Weeties	3/4 cup
Fruit 'n' Nut Weeties	1/2 cup
Shredded Wheat	1 biscuit
Fibre Plus	1/4 cup
Oat Bran	1/4 cup
Cruncy Oat Bran	
with Fruit	1/3 cup
Muesli Flakes	1/2 cup
Natural Apricot &	
Almond Muesli	1/4 cup
Vita Brits	1 biscuit
Rolled Oats	1/4 cup (raw)

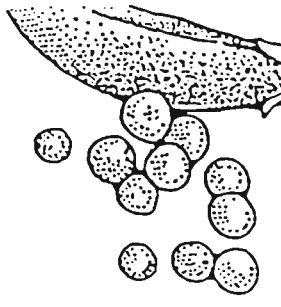
WEIGHT WATCHERS

All Natural Muesli	1/4 cup
All Natural Fruit	
& Fibre	1/4 cup

DIETARY GUIDELINES:

NON INSULIN DEPENDENT DIABETES MELLITUS (NIDDM)

DIET CONTAINING MONOUNSATURATED FAT



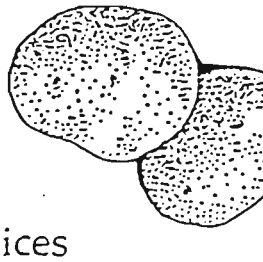
FRUIT, VEGETABLES & LEGUMES:

* Fresh fruit, vegetables and legumes are high in fibre and low in fat.

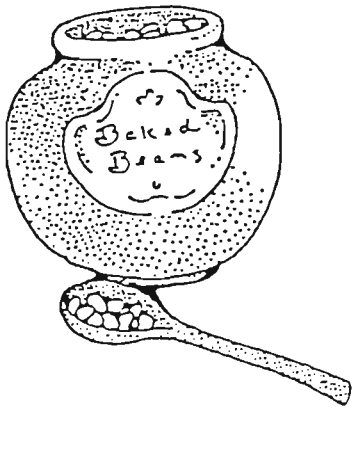
* Include a variety of fruit daily (raw or cooked)

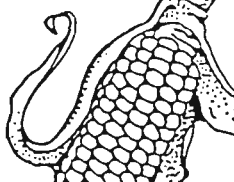
* Include plenty of vegetables daily (especially green and root vegetables)

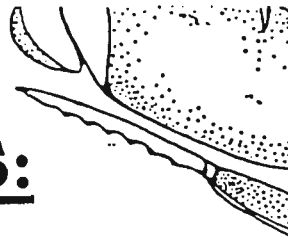
* Legumes include beans (e.g. baked, kidney, soya, lima and haricot) lentils, split and chick peas. Try to include these weekly as an alternative to meat or in meat dishes.
3/4 cup cooked legumes = 75 grams cooked lean meat



* If using oil in the preparation of vegetables use **monounsaturated** oil (for amounts and recommended choices refer to information under "Fats and Oils" in the following pages)





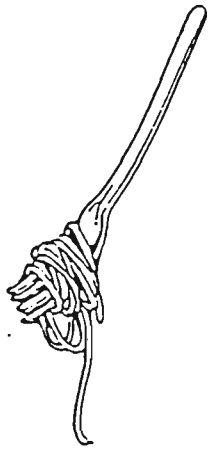


BREADS, CEREALS & GRAINS:

* In their simplest form these foods are all low in fat, and supply dietary fibre, energy, B vitamins and minerals.

* Try to include at least one serve at each meal from the following list of recommended choices.

* *Recommended choices:*



- bread and bread rolls (especially wholemeal varieties)
- pasta (especially wholemeal) **(avoid creamy sauces)**
- rice (especially brown) **(avoid fried rice)**
- crispbreads (Ryvita/ Vitawheat)
- wholemeal English muffins and crumpets (if using margarine spread with monounsaturated margarine .... refer to recommended choices)
- Whole grain cereals (refer to list of best cereal choices) **(avoid toasted and oven baked muesli)**

MILK PRODUCTS:

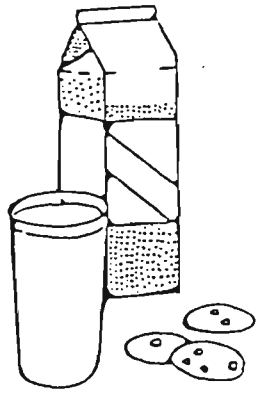
* Dairy products provide calcium, protein, vitamins and energy.

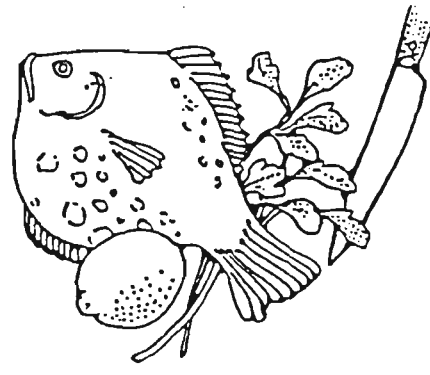
* Include low fat and reduced fat dairy products daily to ensure adequate calcium intake.

Recommended choices:

- Farmers Best milk (is reduced fat and contains monounsaturated fat) *(1st choice)*
- Shape milk (high calcium and low fat)
- Lite White (reduced fat)
- Hi Lite (reduced fat and high calcium)
- Hi Lo (reduced fat)
- Skim milk (low fat)
- reduced fat UHT milk (various brands)
- Diet yoghurt (low fat, artificially sweetened)
- Reduced and low fat cheeses (eg: Devondale 7, cottage, ricotta, Bega super slims, Bega Light ..... plus many more reduced fat varieties).

Farmers Best.





MEAT:

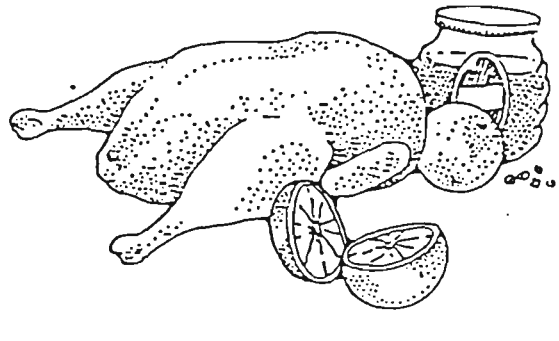
* Meat is a good source of protein, energy, vitamins and iron.

* Include poultry (skin removed) and fish (especially herring, gemfish, ocean perch, red salmon and kippers) a few times a week as the flesh contains monounsaturated fats.

- 1 serve of poultry = 90g
- 1 serve of fish = 150g

* If preparing meat with oil use monounsaturated oil.

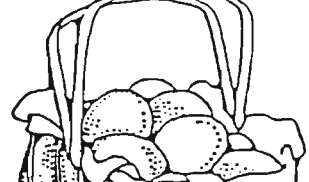
* Include a small serve (75-100g) of lean red meat only a few times a month as it contains saturated fat.



EGGS:

* Maximum of two whole eggs per week (best cooking methods are poaching, boiling, scrambling, microwaving) **(avoid frying eggs).**

* Egg whites may be used instead of whole eggs.



FATS, OILS AND NUTS:

* Fats and oils provide energy and some vitamins.
* Some nuts contain monounsaturated fats, with the added benefit of fibre. They should be eaten in **small** amounts as they are high in kilojoules.
* Some sources of monounsaturated fat should be included daily. They must be used in **limited** amounts as they are **high in kilojoules**, which may result in weight gain.
* Avoid butter and cream **altogether**, and margarines and oils that are **not** monounsaturated.

* **Include no more than _____ serves of fats and oils per day**

* 1 serve = 1 teaspoon Canola margarine
  = 1 teaspoon Canola oil or Olive oil
  = 1 teaspoon Canola mayonnaise
  = 1 teaspoon peanut butter
  = 1 tablespoon avocado
  = 10 olives

* 2 serves of **nuts** = (equal to 2 fat serves)

14 almonds
5 macadamias
10 pecans
10 hazelnuts
10 cashews
20 peanuts
15 PISTACHIO

FOODS TO CONSUME FREELY (in addition to fruit and vegetables)

SPECIAL DIET PRODUCTS:

- diet topping
- diet/low joule cordials
- diet soft drinks
- low joule jelly
- low joule jam/marmalade
- artificial sweeteners

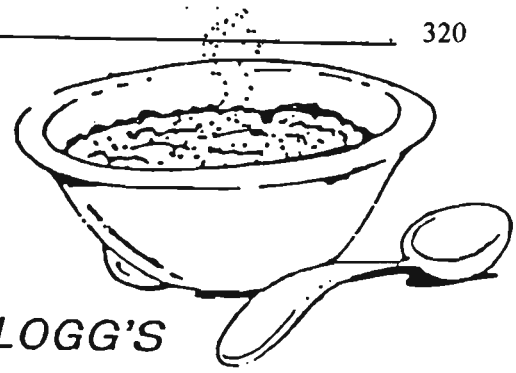
DRINKS

- soda water
- unflavoured mineral water
- unsweetened tomato juice (in moderation)
- tea/coffee

MISCELLANEOUS:

- herbs
- spices
- vinegar
- tomato paste
- garlic
- ginger
- stock cubes
- cooking essences
- curry powder
- lemon juice
- mustard
- Vegemite (although high in salt)

BEST CEREAL CHOICES



These are all low in fat, low in sugar and are good sources of fibre.

SANITARIUM

Weet-Bix	)
Bran Bix	) 2
Lite-Bix	) biscuits
Weet-Bix Hi-Bran	)
Weet-Bix plus Oat Bran	1
Good Start	1
Natural Muesli	1/4 cup
Light 'n Tasty	3/4 cup
Puffed Wheat	1 cup

PURINA

Swiss Formula Muesli	1/4 cup
Fruit 'n' Bran Flakes	1/2 cup

FARMLAND

High Fibre	
Breakfast Bran	1/3 cup
Rolled Oats	1/4 cup (raw)
Minute Oats	1/4 cup (raw)

WILLOW VALLEY

Multi Bran	1/3 cup
Crunchy Toasted Oat Bran	1/4 cup
Barley-O-S	2/3 cup

CEROLA

Oat Bran Muesli	1/4 cup
Natural Muesli	1/4 cup

KELLOGG'S

All Bran	1/4 cup
Bran Flakes	1/2 cup
Ready-Wheats	13 biscuits
Sultana Bran	1/2 cup
Sustain	1/3 cup
Puffed Wheat	3/4 cup
Balance Oat Bran Flakes	2/3 cup
Mini - Wheats	12 biscuits
Mini-Wheats apricot or blackcurrant	10 biscuits

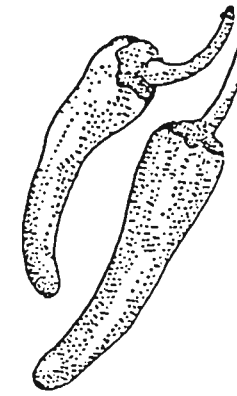
UNCLE TOBY'S

Weeties	3/4 cup
Fruit 'n' Nut Weeties	1/2 cup
Shredded Wheat	1 biscuit
Fibre Plus	1/4 cup
Oat Bran	1/4 cup
Cruncy Oat Bran with Fruit	1/3 cup
Muesli Flakes	1/2 cup
Natural Apricot & Almond Muesli	1/4 cup
Vita Brits	1 biscuit
Rolled Oats	1/4 cup (raw)

WEIGHT WATCHERS

All Natural Muesli	1/4 cup
All Natural Fruit & Fibre	1/4 cup

PRACTICAL TIPS FOR A DIET CONTAINING MONOUNSATURATED FAT

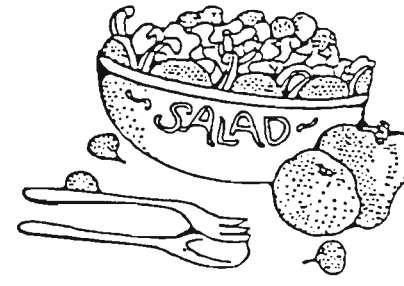


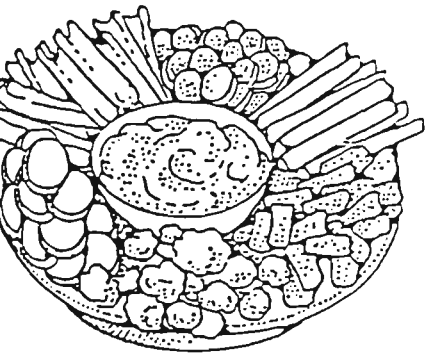
1) Replace polyunsaturated margarines and oils with monounsaturated margarine and oil (eg: Canola, olive):

* Use monounsaturated **margarine** in amounts specified in the diet sheets.

* Monounsaturated **margarine** can be used as a spread on bread and crackerbreads.

* Monounsaturated **oil** can be used in moderation:
- to cook stir fry dishes
- as a marinade for vegetables or meat (add garlic, pepper, reduced salt soy sauce, herbs etc.)
- in a salad dressing (add vinegar, garlic, herbs etc)
- in bean dips



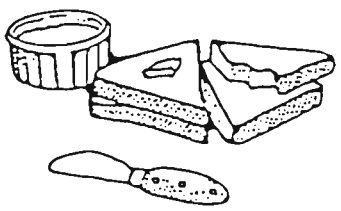


2) Include foods containing monounsaturated fat:

* Choose lean meat, poultry and fish that are high in monounsaturated fats (refer to previous dietary guidelines).

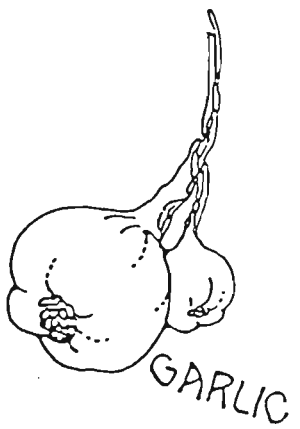
* "Farmers Best" milk contains monounsaturated fat, and is highly recommended.

* Include avocados in recommended amounts as they contain monounsaturated fats. Uses for avocados include:
  - sandwich filling
  - in dips
  - in salads
  - in sauces



* Try to include nuts frequently in recommended amounts (especially macadamias, pecans, almonds, hazelnuts, cashews, peanuts and pistachios as they contain monounsaturated fat and fibre).

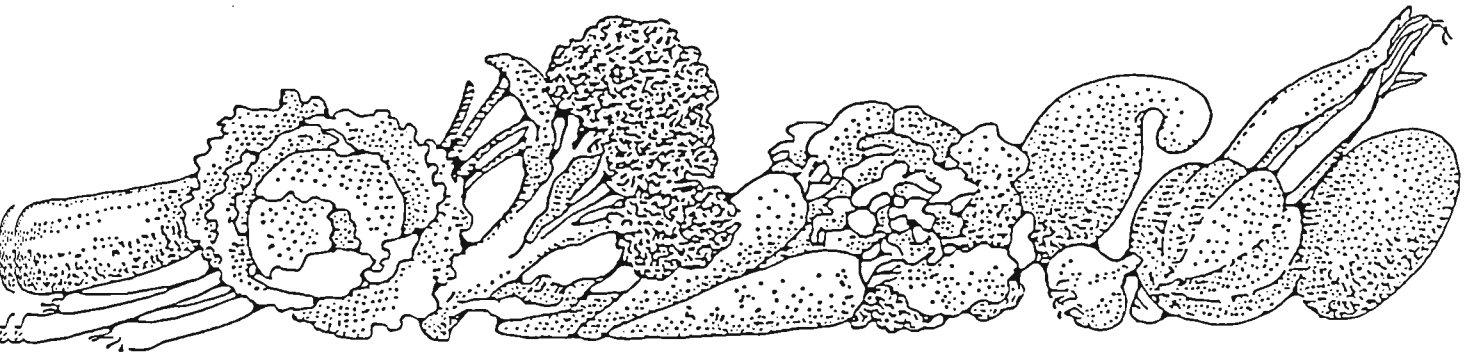
Nuts can be used as:
- a midmeal snack
- in cooking (eg: in meat and vegetable dishes)
- in salads
- as a spread (eg: peanut butter/paste)



3) Maintain an adequate fibre intake:

* Include generous amounts of green vegetables, legumes and whole grain cereals

* Drink plenty of water to prevent dehydration and/or constipation



SUGGESTED MEAL PLAN

BREAKFAST:

cereal/bread

Farmers Best milk

2 monounsaturated fat serves

MORNING TEA:

4 monounsaturated fat serves

(or 2 nut serves)

LUNCH:

4 bread/pasta/rice/potato

4 monounsaturated fat serves

vegetables

fruit

Farmers Best milk

AFTERNOON TEA:

4 monounsaturated fat serves

(or 2 nut serves)

EVENING MEAL:

small serve lean meat (refer to diet sheets)

potato/pasta/rice/bread

vegetables & legumes

5 monounsaturated fat serves

fruit

SUPPER:

2 monounsaturated fat serves

(or 2 nut serves)

diet yoghurt/bread

SAMPLE MEAL PLAN

BREAKFAST:

porridge

Farmers Best milk

2 whole meal toast

2 tsp **Canola** margarine

MORNING TEA:

10 **macadamia nuts**

LUNCH:

2 salad sandwiches made on wholemeal bread & spread with **Canola** margarine

apple

glass **Farmers Best milk**

AFTERNOON TEA:

20 **pecan nuts**

EVENING MEAL:

90g grilled skinless chicken

carrots/peas/beans/broccoli

pasta with 3 tsp olive oil

2 wholemeal bread spread with **Canola** margarine

orange

SUPPER:

1 diet yoghurt with 10 **macadamia nuts** stirred through it

QUESTIONNAIRE HIGH MONOUNSATURATED FAT DIET

1. What were the main problems with the diet?

2. I want to ask about foods now. Have you been able to eat them in the amounts discussed? If you haven't, what were the reasons? Lets start with:

* Bread
* Cereal
* Rice
*Pasta
* Fruit
* Vegetables
* Legumes
* Chicken
* Fish

* Farmers Best Milk _____

_____

* Reduced Fat Dairy Products _____

_____

3. Do you feel that the amount of fat, oil and nuts we suggested you consume was too much, too little or just right?

* Fat _____

* Oil _____

* Nuts _____

- How did you work out how much you were eating?

* Fat _____

* Oil _____

* Nuts _____

- If you haven't been able to have the suggested amount of fat, oil or nuts in your diet why is this?

* Fat _____

* Oil _____

* Nuts _____

- Tell me how you have incorporated fat, oil and nuts into your diet

* Fat _____

* Oil _____

* Nuts _____

4. Have you been able to avoid foods like;

* Polyunsaturated margarine _____

* Full cream dairy products;

- milk _____

- yoghurt _____

- ice-cream _____

- cheese _____

* Cream _____

* Butter _____

* Biscuits _____

* Cake _____

* Chocolate _____

* Chips _____

* Any others _____

- If you weren't able to avoid any of these foods what were the reasons?

_____

_____

_____

_____

5. What different foods are you eating now?

_____

_____

_____

6. Is the palatable and enjoyable?

_____

_____

_____

7. Do you have any food preparation problems?

8. Has cost been a problem with any of the foods? If so, which ones?

9. Has eating away from home or entertaining been a problem? Why?

10. How do you feel about eating like this on a long term basis? Why?

11. Do you have any comments about the information sheets provided?

QUESTIONNAIRE HIGH CARBOHYDRATE DIET

1. What were the main problems with the diet?

2. I want to ask you about foods now. Have you been able to eat them in the amounts discussed? If you haven't what were the reasons?

Lets start with;

* Bread
* Cereal
* Rice
* Pasta
* Fruit
* Vegetables
* Legumes
* Meat
* Reduced Fat Dairy Products

3. Do you think that you are eating fats and oils in limited amounts?

_____

_____

- Why do you think you are (or aren't) eating fats and oils in limited amounts?

_____

_____

_____

4. Have you been able to avoid foods like;

* Full cream dairy products;

- milk _____

- yoghurt _____

- ice-cream _____

- cheese _____

* Biscuits _____

* Cake _____

* Chocolate _____

* Chips _____

* Any others _____

- If you weren't able to avoid any of these foods what were the reasons?

_____

_____

_____

_____

5. What different foods are you eating now?

6. Is the diet palatable and enjoyable?

7. Do you have any food preparation problems?

8. Has cost been a problem with any of the foods? If so, which ones?

9. Has eating away from home or entertaining been a problem? Why?

10. How do you feel about eating like this on a long term basis?

11. Do you have any comments about the information sheets provided?

12. Do you think any other information needs to be provided on these sheets?

13. Can you tell me anything else to help us in understanding what it is like to be on this diet?

FOOD SUBSTITUTIONS MADE USING THE DIET 3 DATA BASE.

FOOD:	DIET 3 ALTERNATIVE:
Farmers Best milk	skim milk + 1g olive oil per 100ml
Canola margarine	olive oil
Canola oil	olive oil
Nan bread	Pita bread
Cup a Soup	soup, light vegetable canned
Diet Lite yoghurt	yoghurt, low fat, plain/natural
crepe	pancake, commercial
rice cake	snacks, cracker, rice/prawn
Lavash bread	Lebanese bread
muesli bar	confectionery bars, fruit
Foccacia bread	white bread roll + 0.5 tsp olive oil
fruit muffin (cake type)	cake, rock, commercial
Diet Coke	soft drink, low energy

FOOD RECORD

PLEASE RECORD EVERYTHING THAT YOU EAT AND DRINK FOR 2 WEEKDAYS AND 2 WEEKEND DAYS.

DAY:		**DATE:**	
TIME OF DAY	TIME	DESCRIPTION OF AMOUNT FOOD OR DRINK	HOW PREPARED / COOKED
BREAKFAST MORNING TEA / SNACKS			

FOOD RECORD

DAY:		**DATE:**	
TIME OF DAY	TIME	DESCRIPTION OF AMOUNT FOOD OR DRINK	HOW PREPARED / COOKED
L U N C H A F T E R N O O N T E A / S N A C K S			

FOOD RECORD

DAY:		DATE:	
TIME OF DAY	TIME	DESCRIPTION OF AMOUNT FOOD OR DRINK	HOW PREPARED / COOKED
D I N N E R S U P P E R / S N A C K S			

FOOD RECORD

DAY:		DATE:	
TIME OF DAY	TIME	DESCRIPTION OF AMOUNT FOOD OR DRINK	HOW PREPARED / COOKED
OTHER TIMES			

DIET ACCEPTABILITY

Code: **Date:**

Surname: **Name:** **Score of 0 to 10**

1. Ease of preparation: 0= Very Difficult 10= Very Easy ---------

2. Cost: 0= Very Expensive 10= Very Cheap ---------

3. Taste: 0= Not tasty 10= Very Tasty ---------

4. Satisfy: 0= Not filling 10= Very filling ---------

5. Variety: 0= Not much Variety 10= Huge Variety ---------

6. Adherence to the diet: 0= Very bad 10= Very good ---------

7. Convenient for the family: 0=Not at all 10=Very convenient ---------

Comments:----------