ASSESSMENT OF THE NUTRITIONAL PROPERTIES OF REGULAR AND LOW LINOLENIC ACID CANOLA OIL IN NON-INSULIN-DEPENDENT (TYPE II) DIABETES MELLITUS SUBJECTS

BY

KATHLEEN MAILIE CHAMBERS

A Thesis
submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of

MASTER OF SCIENCE

Department of Foods and Nutrition
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FACULTY OF GRADUATE STUDIES

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ABSTRACT

The present study was designed to assess the effects of regular and low-linolenic acid canola oil on the blood lipid parameters of 40 (20 men and 20 women) non-insulin-dependent diabetes mellitus subjects, to determine whether canola oil has nutritional benefits in the clinical treatment of NIDDM, with particular attention to any benefits of linolenic acid (LNA). The study also assessed the effects these oils on the oxidative susceptibility, fatty acid composition, and particle size of the LDL fraction.

Difficulty in subject recruitment resulted in a total of 36, newly diagnosed, NIDDM subjects (16 men and 20 women) who were randomly assigned to four experimental groups: regular canola oil (CAN); low-linolenic acid canola oil (LLNA); sunflower oil (SNFLR); and a control (CONT) group who continued their usual diet. Added dietary fat sources were supplied in the form of a margarine spread and salad oil which subjects were counseled to substitute for the usual spread and salad oil in a low-fat (30% total energy), high-carbohydrate (CHO) diet. The average percent of total energy from fat for all subjects was 33.8 ± 2.4%. The percent of the daily fat intake from dietary spread and oil was only 13.4 ± 7.8%, that is 4.5% of total energy.

Twelve-hour fasting blood samples were taken at 0, 28, and 56 days. Plasma total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), and triglyceride (TAG) concentrations were determined on all samples and the LDL fraction was isolated by density gradient centrifugation.

There were no significant effects of diet on plasma TC, LDL-C, HDL-C, or TAG concentrations during any of the study intervals (0-56, 0-28, or 28-56 days, respectively). However, diet did have a significant effect on the TC:HDL-C ratio during the 28-56 day study period; there was a significant decrease in the ratio for the CONT group while there were no changes for the experimental groups. A diet x gender interaction also was observed for the following parameters: LDL-C (0-56 days), HDL-C (0-28 days), and TC:HDL-C (0-28 days). There is no obvious explanation for these observed interactions.

Similarly, there were no diet effects for LDL oxidation rate or the total amount of conjugated dienes produced over the entire experimental period (0-56 days). By contrast, a significant diet effect was observed on the change in LDL oxidation rate during the study period 28-56 days; LDL oxidation rate increased for the SNFLR group, decreased for the LLNA and CONT groups, and remained unchanged for the CAN group. In addition, there was a significant diet x gender interaction for LDL oxidation rate during the study period 0-28 days. Diet had no effect on the change in the amount of conjugated dienes produced during the study periods 0-56 and 28-56 days. However, a significant diet effect was observed during the 0-28 day study period; there was an increase in the amount of conjugated dienes produced on the CONT diet, while negligible changes occurred on the other experimental diets.

Diet x gender interactions were also observed for changes in the levels of the following LDL fatty acids: C18:0 and C20:4 (0-56 day period) and C20:4 (0-28 day period). There is no explanation for these findings. The marginal effects of diet on LDL

oxidative susceptibility is consistent with the relative absence of diet effects on LDL fatty acid composition.

Significant gender differences occurred for all LDL particle size parameters over the 0-56 day study period; LDL core to surface volume ratio (cor/sur ratio), radius, and diameter increased significantly more for men than for women. These results tend to support the reported relationship between TAG levels and LDL particle size. Changes in TAG levels differed significantly between men and women over the study period 0-28 days; TAG levels increased for men and decreased for women.

The present study: (1) demonstrated that regular and low LNA canola oil have similar effects on the blood lipid parameters of NIDDM subjects; (2) confirms earlier research in our laboratory suggesting there are no unique or special benefits of regular canola oil over low LNA canola oil; and (3) provides evidence suggesting that imposing any significant changes on the blood lipid parameters in NIDDM patients is difficult when limited by the restriction of a low-fat diet, namely 30% total energy as fat.

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LIST OF ABBREVIATIONS

Arachidonic Acid	AA
Body Mass Index	BMI
Canola Oil Diet	CAN
Carbohydrate	CHO
Control Diet	CONT
Core to Surface Volume Ratio (LDL Particle)	Cor/Sur
Coronary Heart Disease	CHD
Diabetes Education Centre	DEC
Docosahexaenoic Acid	DHA
Duncan's Multiple Comparison Test	DMCT
Eicosapentaenoic Acid	EPA
Gas-Liquid Chromatography	GC
Glycosylated Hemoglobin	GlyHb
High Density Lipoprotein-Cholesterol	HDL-C
Health Sciences Centre (Winnipeg, MB)	HSC
Linoleic Acid	LA
Linolenic Acid	LNA
Lipoprotein	LP
Low Density Lipoprotein	LDL
Low Density Lipoprotein-Cholesterol	LDL-C

ABBREVIATIONS (Cont'd)

Low Linolenic Acid Canola Oil Diet	LLNA
LDL Particle Radius	r
LDL Particle Diameter	dia
Lipoprotein Lipase	LPL
Monounsaturated Fatty Acids	MUFA
Non-Insulin-Dependent (Type II) Diabetes	NIDDM
Oleic Acid	OA
Oral Hypoglycemic Agents	OHA
Oxidized Low Density Lipoprotein	oxLDL
Palmitic Acid	PMA
Pentadecaenoic Acid	C15:0
Phosphate Buffer Saline (pH 7.4)	PBS
Phospholipids	PL
Protein	PRO
Polyunsaturated Fatty Acids	PUFA
Saturated Fatty Acids	SFA
Stearic Acid	STEA
Sunflower Oil Diet	SNFLR
Total Cholesterol	TC
Total Cholesterol: High Density Lipoprotein-Cholesterol Ratio	TC:HDL-C

ABBREVIATIONS (Cont'd)

Triglycerides	TAG
Tukey's Multiple Comparison Test	TMCT
Unsaturated Fatty Acids	UFA
Very Low Density Lipoprotein-Cholesterol	VLDL-C

LITERATURE REVIEW

I INTRODUCTION

Non-insulin-dependent diabetes mellitus (NIDDM) accounts for approximately 90% of diabetic patients (Sacks and McDonald, 1996) and its prevalence increases with age with approximately half of all cases found in individuals over 55 years. The most important cause of morbidity and mortality in the diabetic population is premature atherosclerosis-related disease, or coronary heart disease (CHD). NIDDM patients are at two to four times the risk of developing CHD as the normal population (Panzram, 1987; Pyorala et al, 1987). Dyslipidemia is an important determinant in the development of CHD in NIDDM patients and is linked to the core characteristics of hyperglycemia and hyperinsulinemia found in the disease. Dietary treatment plays an important role in the management of NIDDM; both the amount and type of dietary fat are closely associated with lipid metabolism and development of CHD. Hence, current dietary recommendations are for a low-fat (30% daily energy), high-CHO (55% daily energy) diet with the primary objective directed at lowering plasma lipids, and in turn, the morbidity and mortality associated with cardiovascular changes and CHD. Research suggests that this diet is effective at lowering plasma total and LDL cholesterol levels in both normolipidemic and NIDDM individuals (Howard, 1987). However, additional evidence supports the findings of concomitant impairment of glycemic control, decreased pre-existing low HDL-C levels, and exacerbation of the hypertriglyceridemia

accompanying NIDDM. The low-fat, high-CHO diet recommendations have been challenged by researchers (Coulston et al, 1989; Grundy, 1991; Parillo et al, 1992) who have shown that a high intake of MUFA is more effective in normalizing glycemic control and blood lipid patterns than a low-fat, high-CHO diet. These researchers also suggest that LDL-C concentrations are within the normal range (1.68-3.40 mmol/L) in NIDDM and that the goal to reduce LDL-C concentrations in these patients misses the point regarding control of more important features characterizing NIDDM and its associated increased risk of CHD.

II PATHOGENESIS OF NON-INSULIN-DEPENDENT DIABETES MELLITUS

The pathogenesis of NIDDM is complicated due to the fact that hyperglycemia and obesity can be both causes and consequences of insulin resistance. Non-insulin dependent (Type II) diabetes mellitus is a complex group of disorders of CHO and lipid metabolism characterized by hyperglycemia and hyperinsulinemia (Figure 1). Hyperglycemia is caused by a combination of hepatic insulin-resistance, impaired peripheral glucose uptake (muscle and adipose), and a defect in glucose-mediated insulin secretion (Proietto et al, 1995). Obesity, particularly abdominal obesity (Sosenko et al, 1993), has been linked to the development of NIDDM (Bjorntorp, 1992). Obesity results in increased plasma free fatty acid levels with the consequent development of muscle and liver insulin-resistance (Boden, 1996). This results in hyperglycemia which then causes a defect in insulin secretion and a worsening of peripheral insulin-resistance via glucose toxicity. The link between obesity and NIDDM can help explain the improvement in

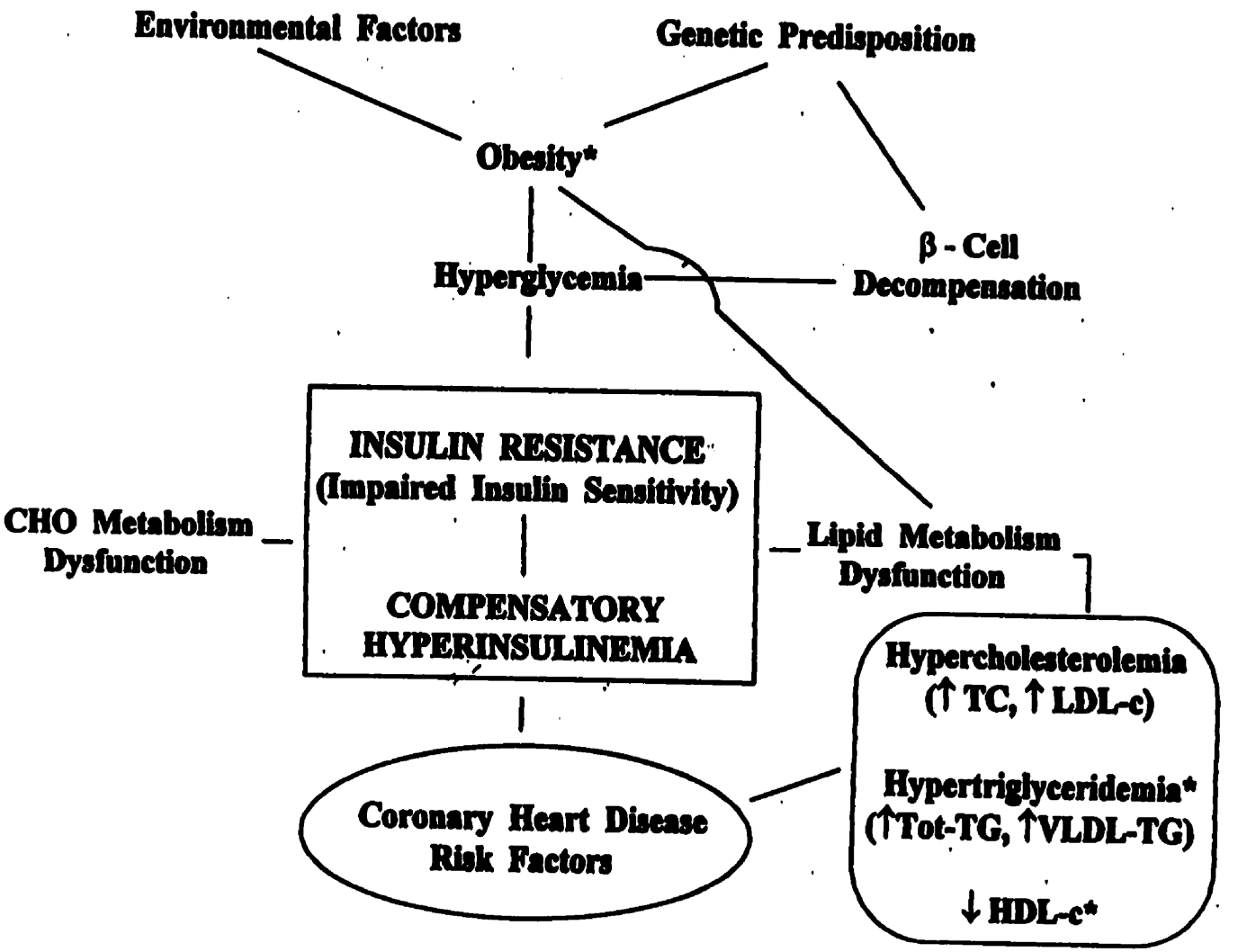


Figure 1. Key Characteristics of NIDDM

hyperglycemia that accompanies weight loss in NIDDM (Proietto et al, 1995). Cohort studies have also consistently found that diabetic women lose their protection from CHD risk (Barrett-Connor and Wingard, 1983; Jarrett et al, 1982; Kannel and McGee, 1987) which may be due to the association between insulin-resistance and hyperandrogenism in these women (Barrett-Connor et al, 1991). Obesity and NIDDM are both independently associated with insulin-resistance (Boden, 1996; Campbell and Carlson, 1993). Obese and NIDDM individuals may have greater insulin-resistance and tend to require more insulin to achieve glycemic control than their lean counterparts (Chung et al, 1995). As stated previously, obesity results in an increased free fatty acid (FFA) level with the consequent development of muscle and liver insulin-resistance (Boden, 1996). The mechanism for this effect is unknown, however, it results in hyperglycemia which then causes a defect in insulin secretion and a worsening of peripheral insulin-resistance via glucose toxicity. However, in the NIDDM population, 80 to 85 % are obese and many experience this effect (Reaven et al, 1988). NIDDM is recognized as a polygenic, extremely heterogeneous, metabolic syndrome with a complex multifaceted pathology. NIDDM is a combination of polygene defects and environmental factors of which hyperglycemia is one element, among others, contributing to development of CHD.

Defects in insulin action and secretion produce metabolic derangement in both CHO and lipid metabolism that are responsible for the disease symptomology. Prospective studies show the increased incidence of CHD in NIDDM patients is not accounted for by the levels of the four main risk factors identified for the general population: age, smoking, blood pressure, and plasma cholesterol levels (Ducimetiere et

al, 1981; Fuller et al, 1983; Kannel and McGee, 1979). These risk factors had similar predictive power in both NIDDM and non-diabetic subjects and the relative CHD risk associated with NIDDM remained significantly higher when adjusted for the four main factors. Therefore, it has been hypothesized that there is something specific about NIDDM which explains the higher risk of CHD and that risk predictors in an already 'at risk' subgroup may differ from the factors which actually contributed to putting the subjects in this high-risk group (Fontbonne, 1991). Blood glucose levels were found not to have predictive power, independent of the four major factors, in predicting CHD risk in NIDDM patients.

Glucose regulation is achieved mainly by the pancreatic hormones glucagon and insulin. Insulin promotes uptake and utilization of glucose by peripheral tissues and is directly related to insulin sensitivity. The main target cells (tissues) for insulin include adipocytes (adipose tissue), skeletal myocytes (skeletal muscle), and hepatocytes (liver) (Zawalich and Kelley, 1995). The main tissues in which insulin-resistance occurs are muscle and adipose tissue (Stout, 1996). Insulin-resistance is defined as a decreased biological response to a normal amount of insulin (Flier, 1992) and data support the concept that insulin-resistance is the primary underlying defect in NIDDM (Kahn, 1994). Two major pathological defects occur in NIDDM: (1) a decreased ability of insulin to act on peripheral tissues (insulin-resistance); and (2) beta (β)-cell dysfunction which is the inability of the pancreas to produce sufficient insulin to compensate for the insulin - resistance. The primary defects in insulin-resistance and secretion are caused by a combination of genetic and environmental factors which, combined, contribute to the

pathology of NIDDM. Hyperinsulinemia has been shown to have a causal role in atherogenesis (Stout, 1996) and high insulin levels are associated with large vessel disease in diabetics (Ronnemaa et al, 1991; Uusitupa et al, 1990). Increasing evidence shows insulin to have activities in a number of processes involved in the development of atherosclerosis (Stout, 1996). Insulin has biological activity in arterial tissue including smooth muscle cell proliferation (Stout et al, 1975) and migration (Nakao et al, 1985), as well as enhancement of lipoprotein metabolism (Oppenheimer et al, 1989; Young and Stout, 1987) all of which provides a possible mechanism for the causal association between insulin and atherogenesis. Pancreatic β-cell deterioration has been shown to progress with increased hyperglycemia leading to failure of the pancreas to produce insulin. This phenomenon is referred to as glucose toxicity (Proietto et al, 1995).

(a) Diabetic Macroangiopathy

The term diabetic macroangiopathy has been used to represent both non-atherosclerotic changes in larger blood vessels as well as large vessel disease occurring in NIDDM individuals in the form of atherosclerosis development. Jens et al (1996) have proposed the concept of a specific diabetic macroangiopathy hypothesis to replace the usual theory of a link between atherosclerosis and NIDDM. Their hypothesis suggests a causal relationship between changes, which occur in the NIDDM disease state independent of the presence of atherosclerosis in the large vessels of NIDDM individuals, and the abnormal metabolism in NIDDM. This research group believes their theory will lead to a better working strategy for further research, prevention, and treatment of

NIDDM. Support for this perspective comes from earlier research by Jarrett (1984) in which it was noted that cardiovascular complications were as common in newly diagnosed diabetics as in those whose diabetes was of long duration. The researchers involved in the study reasoned that if CHD were a complication of NIDDM, then its frequency would increase with increased duration of diabetes. However, this correlation was not observed in the Whitehall study (Jarret and Shipley, 1988) in which non-diabetic subjects who were at increased risk of CHD due to impaired glucose tolerance seemed to have the same rate of CHD as those with long-established NIDDM. Hence, they speculated that CHD, rather than a complication of NIDDM, shares environmental and genetic elements with NIDDM.

(b) Insulin-Resistance and Its Relationship to Coronary Heart Disease in NIDDM

The effects of insulin are well understood, however, the mechanism of action is not well defined. Insulin-resistance was first noted to be involved in the pathogenesis of NIDDM by Ginsberg et al (1975) and Harano et al (1981) and has since been shown to predict development of NIDDM (Haffner et al, 1992). Insulin-resistance, especially as it affects fat cell lipid metabolism, modulates many steps involved in lipoprotein transport of cholesterol and TAG, hence playing a major role in the dyslipidemia and increased CHD found in NIDDM (Ginsberg, 1996). The clinical spectrum of insulin-resistance in NIDDM is quite broad. Indirect measurements of insulin function include fasting insulin concentration or the insulin response to glucose. One of the most important clinical syndromes associated with insulin-resistance is called 'Syndrome X' (Haffner et al, 1992)

in which patients exhibit resistance to insulin, hyperinsulinemia, and lipid abnormalities including increased VLDL, total TAG, low HDL-C, abdominal obesity, and hypertension (Haffner et al, 1992; Reaven, 1988; Stern, 1996; Suzuki et al, 1996). All of these features are associated with increased incidence of CHD and have been established as risk factors for development of the NIDDM syndrome. Hence, all of the elements of insulin resistance syndrome are risk factors, and usually key characteristics, of NIDDM. Despite this link, there has been little impact of this finding on the treatment and care of NIDDM patients (Kahn, 1994).

Several elements of the insulin-resistance syndrome are known CHD risk factors including abdominal obesity (Hubert et al, 1983; Larsson et al, 1984), hypertension (Keys et al, 1971), and low HDL-C levels (Gordon et al, 1989). An insulin-resistant state at the cell level has been shown to have atherogenic potential in that it is consistently accompanied by other metabolic disturbances including dyslipidemia (hypertriglyceridemia and decreased HDL-C), glucose intolerance, and elevated blood pressure levels. These abnormalities may influence cardiovascular risk through synergistic effects of the single risk factors. Hyperinsulinemia is associated with insulin-resistance in NIDDM and is often associated with hypertriglyceridemia (Steiner and Lewis, 1996). The finding that hyperinsulinemia is a CHD predictor identifies the relationship between insulin-resistance and its accompanying metabolic syndrome, of which hyperinsulinemia is seen as a 'marker' to cardiovascular risk.

The primary defect in CHO metabolism in NIDDM is insulin-resistance at the level of the peripheral tissues (Fuller, 1985). This defect is at the core of the metabolic

syndrome associated with NIDDM and links it to extreme glucose intolerance (hyperglycemia), hyperinsulinemia, and dyslipidemia (Fuller, 1985). The lipid metabolism abnormalities found in NIDDM, including high triglyceride and VLDL-C levels as well as decreased HDL-C levels, are important markers of cardiovascular disease risk in NIDDM. However, its role as a contributing factor in arterial lesion initiation and progression in NIDDM is still unclear. The finding of an association between dyslipidemia and future cardiovascular risk in NIDDM patients reinforces the hypothesis that the metabolic syndrome of insulin-resistance may influence the progression and development of CHD.

Insulin-resistance precedes the full development and diagnosis of NIDDM while hyperinsulinemia, one of the diseases early markers, predicts CHD which may explain the extensive atherosclerotic disease-related damage found at initial diagnosis of NIDDM.

The dyslipidemia of NIDDM has been suggested as a better indicator of severity of insulin-resistance because at the stage of overt diabetes, pancreatic secretion of insulin is altered and tends to decrease continuously over time (DeFronzo, 1988; Saad et al, 1989). High plasma triglyceride concentration is a consistent predictor of future cardiovascular incidence in NIDDM patients and is due to the relation between the metabolic syndrome of insulin-resistance and CHD.

(c) Carbohydrate and Lipid Dysfunction in NIDDM

NIDDM individuals are characterized by hyperglycemia resulting from reduced insulin-sensitivity at the cell tissue level (insulin resistance) and compensatory

hyperinsulinemia; the result of insulin resistance. These two factors represent the major metabolic abnormalities in NIDDM. Diabetes is well known as a dysfunction of CHO metabolism. However, abnormalities in lipid and lipoprotein metabolism are also characteristic of the syndrome. These lipid abnormalities are of great concern given the increased risk and development of CHD found in NIDDM and the effect that dietary amount and type of fat has on lipid metabolism, and hence, CHD. The dyslipidemia present in the NIDDM syndrome is characterized by hypertriglyceridemia and results primarily from increased endogenous triglyceride synthesis (Ginsberg and Grundy, 1982; Greenfield et al, 1980; Kissebah et al, 1974; Nikkila and Kekki, 1973). In NIDDM patients, hypertriglyceridemia is a concern due to its strong relationship to CHD in this population (Fontbonne et al, 1989). A reduction in plasma triglyceride levels in response to improved glycemic control in NIDDM is related to a reduction in VLDL secretion, secondary to a decrease in free fatty acid levels (Greenfield et al, 1980; Kissebah et al, 1974). Some researchers have found that LDL-C concentrations do not differ substantially from non-diabetic individuals (Nikkila, 1984) yet the major goal of dietary recommendations is to reduce LDL-C levels. In addition, low plasma HDL-C levels are frequent in NIDDM patients (Hollenbeck et al, 1986).

(1) *Atherosclerosis Development as Defined by Carbohydrate and Lipoprotein Dysfunction*

Both abnormalities of CHO and lipoprotein (LP) metabolism in NIDDM are related to increased risk of developing CHD. Hypertriglyceridemia has been associated

with increased CHD incidence in cross-sectional and prospective studies (Carlson et al, 1979; Fontbonne et al, 1989; Santen et al, 1972; West et al, 1983). The World Health Organization multinational study (West et al, 1983) and the Paris Prospective study (Fontbonne et al, 1989) emphasize the importance of hypertriglyceridemia as an independent risk factor for CHD in NIDDM patients. The limitations of these two studies are that only total plasma triglyceride and cholesterol concentrations were measured and not the lipoprotein fractions. In addition to hypertriglyceridemia, studies (Castelli et al, 1977; Miller and Miller, 1975) have found low HDL-C levels to be associated with increased CHD risk. Evidence also shows individuals with NIDDM have reduced HDL-C levels which may contribute to the enhanced atherosclerotic-related disease of the NIDDM syndrome (Beach et al, 1979; Reckless et al, 1978). The inverse relationship between VLDL-triglyceride and HDL-C (Havel, 1979) has been suggested to reflect the interchange of cholesterol esters and triglyceride between HDL and VLDL particles, representing a recycling of cholesterol esters from HDL back to VLDL (Nestel et al, 1979).

The role total and LDL cholesterol play in the atherogenesis of NIDDM is an uncertain and debated issue. Increased total and LDL cholesterol are strong CHD risk factors in the general population. Some researchers believe these factors have not been identified as strong risk factors for CHD in the NIDDM population (Hollenbeck and Coulston, 1991), that they are the most normal aspect of lipoprotein metabolism in this group, and that they do no appear to be elevated compared with a normal glucose tolerant population. Conversely, other research suggests there is a relationship (Abbott et al,

1989; Hara et al, 1992). In addition, evidence that elevated plasma insulin levels play a significant role in CHD development has accumulated (Hamsten et al, 1987; Kuo and Feng, 1970; Stout, 1990; Stout and Vallance-Owen, 1969; Zavaroni et al, 1989). Hyperinsulinism is common in NIDDM patients (Coulston et al, 1987; Fontbonne et al, 1989; Santen et al, 1972) and evidence exists that it may play a significant role in the development of CHD in NIDDM (Fontbonne et al, 1989; Santen et al, 1972; Zavaroni et al, 1989).

(d) Lipoprotein Metabolism Dysfunction in NIDDM and its Relationship to the Development of Atherosclerosis

LDL are the major cholesterol carriers in the plasma and are considered to be the most atherogenic lipoprotein particle (Havel and Rapaport, 1995). Evidence confirms the positive and causal relationship between LDL-C and the development of atherosclerosis-related disease (Castelli et al, 1986; Reaven, 1991). Increased incidence of mortality due to CHD associated with elevated LDL levels has been reported by many researchers (Anderson et al, 1987; Martin et al, 1986). In addition, intervention studies, such as the Helsinki Heart Study (Frick et al, 1987) have strongly supported the relationship between elevated LDL-C concentrations and atherogenesis (Stalmer et al, 1993).

Altered lipid metabolism is common in NIDDM patients as insulin plays a key role in lipid and lipoprotein metabolism. The extent of insulin-resistance, hyperglycemia, obesity, and the presence of other primary and secondary causes of hyperlipidemia will

determine the lipid and lipoprotein levels of each individual patient and hence, their risk of developing CHD.

(1) High Density Lipoprotein Cholesterol

Epidemiological studies show HDL-C is strongly and inversely related to CHD; low HDL-C levels are an important predictor of CHD (Gordon et al, 1977; Miller et al, 1977). The protective role HDL-C plays in CHD is explained by its function in reverse cholesterol transport. It serves as an acceptor of cellular free cholesterol which is then transferred to other LP that ultimately reach the liver for excretion (Reichl and Miller, 1986). The subfraction of HDL-C most closely associated with vascular disease, HDL_2, tends to be low in NIDDM (Hollenbeck et al, 1986; Nikkila and Hormila, 1978) and seems to be more strongly related to cardiovascular disease in NIDDM patients than the normal non-diabetic population (Gordon et al, 1977; Reckless et al, 1978). HDL metabolism is related to VLDL metabolism and alterations in lipoprotein lipase (LPL) activity. LPL activity has been shown to be decreased by hyperinsulinemia. Hence, transfer of surface components of triglyceride-rich LP (VLDL) to HDL during LP hydrolysis by LPL is reduced, resulting in low HDL levels and hypertrigyceridemia (Nikkila, 1978). When hypertriglyceridemia and hyperinsulinemia are corrected, HLD-C levels tend to stay low or show only moderate improvement suggesting other factors are involved in producing low HDL-C levels in NIDDM. An inverse relationship between plasma insulin levels and HDL-C, independent of VLDL concentration, has been described (Jiao et al, 1986; Uusitupa et al, 1986). Elevated levels of hepatic lipase

enzyme, important in HDL-C metabolism, may explain decreased HDL-C levels in NIDDM. Other causes of low HDL, including obesity, may coexist in the NIDDM disease state.

(2) Very Low Density Lipoprotein Cholesterol

Very low density lipoprotein (VLDL) is hydrolyzed by LPL to produce the intermediate density lipoprotein particle (IDL) which can be removed by the liver or undergo conversion to LDL. VLDL metabolism in NIDDM patients is altered, and elevated plasma triglyceride levels are common, most likely due to a combination of increased hepatic production and decreased removal of VLDL due to decreased LPL activity (Betteridge, 1986; Howard, 1987).

(3) Triglycerides

Plasma triglyceride (TAG) concentration is known to be an independent risk factor of CHD (Cambien et al, 1986) and an increased concentration of LDL triglycerides is an independent CHD risk factor for NIDDM individuals (Lahdenpera et al, 1996). Elevated TAG concentrations are common in NIDDM and are strongly associated with atherosclerotic-related vascular disease in this population (West et al, 1983; Taskinen, 1992). Risk of CHD related to increased TAG levels is compounded by decreased HDL-C levels in NIDDM. Serum TAG concentration in NIDDM has been shown to correlate with the glycosylated hemoglobin level, and hence glycemic control. However, mild

hypertriglyceridemia and low HDL-C levels usually persist even in well controlled NIDDM (Taskinen et al, 1992).

(4) Low Density Lipoprotein Cholesterol

Low density lipoprotein cholesterol (LDL-C) levels vary according to glycemic control, hence, NIDDM can affect LDL metabolism in two ways. The first is by reducing LDL binding which has been observed in cultured cells grown in serum from poorly controlled diabetic patients (Lopes-Virella et al, 1985). It has been suggested that this may be secondary to cell membrane changes affecting the LDL receptor and leading to delayed LDL clearance. The second way NIDDM can affect LDL metabolism is through glycosylation of the lysine residues of LDL apolipoprotein B secondary to hyperglycemia. Apolipoprotein B plays a crucial role in LDL binding to its receptor. LDL isolated from NIDDM patients have been found to have up to 5% of apoB lysine residues glycosylated; 3 to 4 fold higher than normal (Curtiss and Witztum, 1985; Schleicher et al, 1981). LDL isolated from hypertriglyceridemic NIDDM patients had decreased receptor binding to cultured skin fibroblasts (Hiramatsu et al, 1985) whereas NIDDM patients without hypertriglyceridemia and similar glycemic levels showed normal binding. Improved glycemic control is associated with reduced triglyceride content of the LDL particle (Lopes-Virella et al, 1982). In a recent study (Lahdenpera et al, 1996), LDL was found to be significantly lower in NIDDM subjects compared to the non-diabetic subjects. This study also found NIDDM TAG content to be significantly higher. When assessing CHD risk for NIDDM individuals, it may be more important to

focus attention on the composition of LDL, as it relates to size and oxidizability, rather than the absolute level as LDL-C concentrations in NIDDM usually are normal (Dimitriadis et al, 1995; Ronnemaa et al, 1989).

(i) Effects of Hyperglycemia in NIDDM

The traditional cardiovascular risk factors known to be part of NIDDM do not fully explain the increased morbidity and mortality of this disease. Other factors such as diabetic dyslipidemia and hyperglycemia (Kuusisto et al, 1994; Uusitupa et al, 1993) itself may contribute to the excess CHD in NIDDM. Lipid peroxidation has been associated with poor metabolic control in NIDDM patients (Ceriello et al, 1991). The role of hyperinsulinemia and insulin resistance in the pathogenesis of NIDDM has been studied, however, there are no reports on the relationship of hyperinsulinemia and hyperglycemia to lipid peroxidation in the many different states of glucose intolerance. Oxidative stress has been shown to be increased *in vivo* in the diabetic state and is a result of increased oxygen free radical production as well as a decrease in the scavenging of these molecules (Kashiwagi et al, 1996). The generation of free radicals (e.g. superoxide anion, hydrogen peroxide, hydroxyl radical) has been found (Ceriello et al, 1991; Sato et al, 1979) and is suggested to be increased in the diabetic state (Ceriello et al, 1993; Mullarkey et al, 1990) leading to diabetic complications (Ceriello et al, 1993; Jenning et al, 1987). These free radicals may react with PUFA in biological membranes leading to lipid peroxidation which may in turn increase cell membrane rigidity, affect endothelial function, and modify lipoproteins, hence increasing vascular disease (Halliwell and

Chirico, 1993). Increased lipid peroxidation has also been observed in NIDDM patients when compared to controls (Gallou et al, 1993; Katoh, 1992; Walter et al, 1991). Excess lipid peroxides in the vascular system can induce major endothelial cell dysfunction, including stimulation of production of platelet activating factor resulting in increased vascular permeability, and inhibition of prostaglandin I_2 synthesis resulting in abnormal coagulation (Lewis et al, 1988; Wharton et al, 1985; Zweier et al, 1988). These dysfunctions in endothelial cells may be found in diabetes. It is important to explore other any further mechanisms that result in the increased oxidative stress on the vascular tissue of NIDDM. It is also important to uncover the effects of hyperglycemia on radical scavenger function in endothelial cells (Kashiwagi et al, 1996). With the increase in lipid peroxidation products found in NIDDM being related to plasma glucose and insulin levels, a role for insulin resistance is suggested. Support for this theory comes from the association between lipid peroxidation and elevated serum TAG levels (Stringer et al, 1989); hypertriglyceridemia is a key factor in the pathogenesis of NIDDM and insulin resistance syndrome. Abnormalities in glucose metabolism in endothelial cells may exist and may be related to the pathogenesis of macrovascular complications in diabetes (Baynes, 1991; Brownlee, 1992; Diabetes Control and Complication Trial Research Group, 1993; Schwartz, 1992). Other mechanisms for radical scavenger dysfunction in diabetes have been suggested. Cu, Zn-superoxide dismutase has been shown to be inactivated by the non-enzymatic glycation and oxidation mechanism (Arai et al, 1987). Under diabetic conditions, the antioxidant enzymes, including Cu, Zn superoxide dismutase, are decreased (Taniguchi et al, 1996). Oxidative stress is a result of the

inactivation of this antioxidant enzyme as well as the increased production of reactive oxygen species in the NIDDM state (Taniguchi et al, 1996) . Reduced content of antioxidant substances, including vitamin E and ascorbic acid, are also reported in NIDDM tissues (Kaplan et al, 1982; Yue et al, 1989). These results provide evidence that in the hyperglycemic condition, increased oxygen free radical production and decreased degradation of superoxide anion and hydrogen peroxide, may lead to deterioration of endothelial cell function. This event may be one of the initial steps of diabetic vascular dysfunction. The clinical significance of these abnormalities regarding the pathogenesis of diabetic complications requires further research. Evidence of increased oxidative stress in NIDDM, including increased levels of peroxidation of plasma lipid (Morel and Chisolm, 1989; Sato et al, 1979) and red cell membrane (Jain, 1989), impaired release of endothelium-derived relaxing factor (Tesfamarian and Cohen, 1992), and autoxidation products (Bucala et al, 1993; Wolff and Dean, 1987) has accumulated. It has been suggested that normalization of these conditions be taken into account when treating NIDDM individuals (Kashiwagi et al, 1996).

(ii) LDL Glycation: Its Effect on LDL Oxidation

Non-enzymatic glycosylation is one pathway of glucose disposal implicated in the pathogenesis of NIDDM CHD complications (Pugliese et al, 1991). It has been suggested that glycosylated hemoglobin levels are a univariate predictor of CHD end points in NIDDM subjects (Laakso et al, 1993). A mechanism explaining the role of

hyperglycemia in atherogenesis has been proposed by Bucala et al (1993) in which advanced glycation end-product modification of LDL has resulted in its decreased clearance in transgenic mice expressing the human LDL receptor (Bucala et al, 1994). Elevated circulating levels of this type of modified LDL have been shown in NIDDM (Bucala et al, 1993) and were lowered by administration of an advanced glycosylation inhibitor (Bucala et al, 1994). The diminished clearance of the modified LDL could contribute to diabetic dyslipidemia, hence providing a mechanism linking hyperglycemia to atherosclerotic cardiovascular disease in NIDDM. It is of particular importance that the apoB protein of LDL-C undergoes non-enzymatic glycation involving the formation of a stable bond between glucose and a protein amino group (Sobenin et al, 1996). Modification of LDL in this way has been reported to reduce peripheral receptor-mediated LDL uptake (Lorenzi et al, 1984; Witztum et al, 1982), thus making more lipoprotein available for non-receptor-mediated permeation of vascular walls and scavenger receptor uptake by macrophages. LDL from NIDDM was characterized by increased non-enzymatic glycation by Sobenin et al (1993). The potential pathophysiological significance of advanced glycation end-products results from the association of their accumulation in tissues, the vessel wall, and plasma to the pathogenesis of NIDDM complications (Schmidt et al, 1996). Receptors for these glycation products have been shown to exist and be expressed by critical cell types involved in the pathogenesis of NIDDM, including endothelial cells, mononuclear phagocytes and smooth muscle cells (Horiuchi et al, 1996) LDL glycation has also been shown to be related to LDL oxidizability (Hunt et al, 1990). However, the increase in

glycated apoB levels in NIDDM was not dramatically high and sometimes was not observed (Kortlandt et al, 1992), and the atherogenic effect of these products is therefore believed to be very moderate.

The relationship between increased lipid peroxidation and metabolic control in NIDDM individuals is not well understood as studies report conflicting results. Whether the hyperglycemia and hyperinsulinemia characteristic of NIDDM are related to lipid peroxidation is unknown at present. Hyperglycemia in NIDDM may induce large increases in glycated products (Sobenin et al, 1996). However, Niskanen et al (1995), report that glucose tolerance status is the major determinant of increased lipid peroxidation even after controlling for the effects of age, gender, BMI, physical activity, smoking, and diuretic use. In this study, the major determinants of lipid peroxidation were fasting plasma glucose, insulin, and apoA (inverse relationship) levels which suggests a role for insulin-resistance and hyperglycemia in the increased lipid peroxidation of NIDDM due to increased overall oxidative stress (Taniguchi et al, 1996). Increased lipid peroxidation has been suggested to reflect a part of the continuous cycle of oxidative stress and damage that result in NIDDM CHD complications (Baynes, 1991). It has been reported that oxidative stress is increased *in vivo* in the NIDDM state (Kashiwagi et al, 1996). Glycation of various proteins in the NIDDM state is a result of attachment (covalent) of glucose to protein amino groups, resulting in changes in surface charge, hydrogen bonding capacity, cellular and receptor recognition, and/or formation of complex products (Niskanen et al, 1995). No studies have directly proven the role of insulin-resistance in lipid peroxidation. However, lipid peroxidation has previously been

associated with elevated TAG levels (Stringer et al, 1989) and hypertension (Tagami et al, 1992) which are both characteristic features of the insulin-resistance syndrome of NIDDM.

(e) LDL Oxidation and LDL Particle Size in NIDDM

Type II diabetics have standard as well as non-traditional risk factors which predispose them to increased risk of CHD. The first of two major non-traditional risk factors is oxidation of lipoproteins, specifically LDL lipoprotein, which plays an important role in atherosclerosis development. The second risk factor in CHD development for NIDDM individuals is the presence of small, dense LDL particles which may have increased atherogenic effects as they enter the arterial wall more readily and are more easily oxidized (Reaven, 1995). These two factors are of particular importance due to the greater rates of lipid oxidation *in vivo* that may exist in NIDDM individuals when compared to non-diabetic individuals, along with evidence of increased prevalence of small, dense LDL particles in NIDDM.

Certain dietary treatments have been suggested to reduce *in vivo* lipid peroxidation and susceptibility of LDL and dense LDL subfractions to oxidative modification in NIDDM patients. A reduction of dietary saturated fat in NIDDM individuals has been shown to reduce total cholesterol levels and hence CHD risk. Controversy over the appropriate diet of NIDDM individuals stems from whether dietary saturated fat should be replaced by CHO or unsaturated fatty acids and if unsaturated fat is shown to be superior, should it be replaced by mono- or poly- unsaturated fatty acids?

Recent studies have showed decreased susceptibility to oxidation of LDL in subjects consuming MUFA-enriched diets (Dimitriadis et al, 1995).

(1) Effect of NIDDM on LDL Particle Size

The more common CHD risk factors seen in NIDDM does not completely explain their increased morbidity and mortality from CHD (Wingard et al, 1983). In humans, LDL particles are the major carriers of plasma cholesterol. LDL, as a subfraction of plasma, has a large heterogeneity based on measures of size, density, and chemical composition (Krauss, 1991). Small, dense LDL has been associated with increased risk of myocardial infarction (Austin et al, 1988). Small, dense LDL subclass patterns are more common in NIDDM patients (Haffner et al, 1994; Feingold et al, 1992; Detwart et al, 1992). LDL size parameters have many genetic and environmental influences including gender, age, diet, drugs, obesity, hormones, and diabetes mellitus (Austin et al, 1990; Campos et al, 1991b; Reaven et al, 1993a). Factors associated with decreased LDL particle size are male gender, elevated TAG, and decreased HDL-C levels (McNamara et al, 1987; Swinkels et al, 1989). Also, the prevalence of small LDL particles has been associated with populations consuming low-fat, high-CHO diets (Campos et al, 1991a). Research suggests that TAG-rich, cholesterol-depleted LDL particles have a decreased affinity for the LDL receptor compared with normal LDL (Aviram et al, 1988). This would promote LDL traveling longer in the circulation and increase its exposure to and probability of oxidation; oxidized LDL (oxLDL) is also known as a factor for increased

CHD. One study (de Graaf et al, 1991) indicates that smaller, denser LDL particles from normal plasma are more susceptible to oxidation *in vitro*. LDL particle size is highly associated with total and abdominal fat (Campos et al, 1991b) and has been correlated with exercise in women (Lamon-Fava et al, 1989). Studies indicate that small LDL particles are more prevalent in men with CHD than in controls. These LDL particles have also been associated with a three fold increased risk of myocardial infarction (Austin et al, 1988; Crouse et al, 1985). However, when adjustments are made for TAG levels, these differences are no longer significant. The association between LDL particle size and CHD is not independent when established CHD risk factors have been considered (Campos et al, 1991a). The interrelationships between LDL particle size and lipid and lipoprotein levels, especially TAG and HDL-C levels, and the use of medications, makes the role of LDL particle size in patients with CHD uncertain. Smaller LDL particles are associated with increased TAG and decreased HDL-C levels as well as being associated with diabetes, hypertension, total cholesterol, and apoB levels (Campos et al, 1992b). In the study of Campos et al (1992b), adjusting for TAG and HDL-C together significantly reduced the differences associated with LDL particle size between CHD and control subjects; in fact, the differences were no longer statistically significant. Patients with CHD had significantly higher TAG levels than controls as well as lower HDL-C levels. Further analysis showed that TAG levels and LDL particle size were not independent risk factors (Campos et al, 1992b). TAG levels and LDL particle size are highly correlated and neither reached independent significance in the presence of the more established risk factors in the model (Campos et al, 1992b). Instead, their association with low HDL-C

suggests these parameters reflect a number of alterations in lipoprotein metabolism that increase CHD risk. Whether small LDL particles alone are atherogenic, remains to be elucidated. It seems that LDL size alone cannot determine its atherogenicity but that this factor in combination with its other physical and chemical characteristics is more relevant in determining LDL atherogenicity.

Three measures of LDL particle size are commonly assessed; LDL core to surface volume ratio (Cor/Sur), radius, and diameter. LDL diameter is determined using both LDL cor/sur ratio and radius. Hence, when discussing LDL diameter, all three parameters have been assessed. NIDDM is associated with significantly smaller LDL particle diameter (Singh et al, 1995). However, when LDL diameter measure is correlated with measures of HDL-C, LDL-C, TAG, apo AI, apo B, and apo E concentrations, it has been found that the effect of NIDDM on LDL diameter is of no significance (Singh et al, 1995). Research suggests that the effects of NIDDM on lipoprotein metabolism is the cause of the change in their lipoprotein particle size (Singh et al, 1995).

An atherogenic lipoprotein profile is seen in insulin-resistance syndrome, Syndrome X (DeFronzo and Ferrannini, 1991; Reaven, 1988). This syndrome includes other CHD risk factors of which hypertension, obesity, body-fat distribution, and glucose tolerance are included. Factors associated with decreased LDL particle size include male gender, elevated TAG, and decreased HDL-C levels (McNamara et al, 1987; Swinkels et al, 1989). LDL from diabetic subjects shows increased heterogeneity with a pattern of smaller, B-pattern particles (Reaven et al, 1993a). B-pattern particles have been

described by Austin and Krauss (1986) and Austin et al (1990) as small, dense LDL particles. Subjects exhibiting this pattern of LDL tend to have atherogenic lipoprotein profiles including higher levels of TAG, VLDL and IDL mass and apoB and lower levels of apoA and HDL-C (McNamara et al, 1987). The accumulation of B-pattern particles in NIDDM subjects may depend on changes in TAG metabolism (Feingold et al, 1992). NIDDM HDL-C levels are altered with respect to decreased particle size which may also be related to increased TAG concentrations found in these individuals. Associations between LDL particle size and other existing lipoprotein parameters in NIDDM require clarification.

Small, dense LDL subclass patterns appear to be more frequent in NIDDM (Singh et al, 1995; Barakat et al, 1990; Detwart et al, 1992), an important observation due to the association of smaller LDL particles and the atherogenic LDL subclass pattern B. Lipoprotein abnormalities have been shown to be physiologically linked with insulin action (Abbot et al, 1987). The abnormalities of insulin action, hyperinsulinemia, and insulin-resistance, seen in NIDDM, play an important role in regulation of lipoprotein metabolism and the dyslipidemia found in NIDDM (Haffner et al, 1990; Ferrannini et al, 1991). It has been reported that a decrease in plasma levels of insulin and TAG in NIDDM may result in LDL particle size reversion to the normal state (Barakat et al, 1990) and that this change may reduce CHD risk for NIDDM. Hence, a correlation of insulin and TAG concentrations with diabetic LDL particle size is established. Overall, however, no significant effect of NIDDM on any LDL particle size parameter has been found. Hence, even with substantial effects of NIDDM on certain measures of

lipoprotein sizes, data suggest these effects are on the general metabolic processes as opposed to being specific to particle size (Singh et al, 1995). The unanswered question remains, is NIDDM an independent risk factor for LDL particle size?

(2) TAG and LDL Particle Size in NIDDM

Serum TAG, in healthy subjects, is the major determinant of LDL particle size (Barakat et al, 1990; Coresh et al, 1993; Deckelbaum et al, 1984; Feingold et al, 1992). In NIDDM patients, with or without CHD, serum TAG also was found to be the major determinant of the properties of LDL particles, hence, the influence of NIDDM on particle size is explained by serum TAG concentrations (Lahdenpera et al, 1996). The clinical implication, therefore, is that in NIDDM, serum TAG levels should be as low as possible in order to prevent atherogenic changes in LDL particles (Lahdenpera et al, 1996). Haffner et al (1994) reported that NIDDM has as independent effect on LDL particle size in women but not in men. The diabetic dyslipidemia in men explained the association between small, dense LDL and NIDDM (Haffner et al, 1994). Research suggests hypertriglyceridemia is an independent risk factor for CHD in NIDDM (Fontbonne et al, 1989; Laakso et al, 1993) and that LDL particles in NIDDM are TAG enriched and hence indicate LDL compositional changes (Tilly-Kiesi et al, 1992; Uusitupa et al, 1993). Serum TAG concentration has been shown to be important in determining LDL characteristics (Barakat et al, 1990; Coresh et al, 1993), including LDL particle size. Lahdenpera et al (1996) found TAG concentration to be the major determinant of LDL particle diameter. As well, HDL-C concentration appeared to be an

independent determinant of LDL particle size. Hence, it is not certain that the atherogenic effect of NIDDM is due solely to the hypertriglyceridemia or due to its other metabolic consequences. Small, dense LDL is usually accompanied by high TAG concentrations (Landenpera et al, 1996) and decreased HDL-C concentrations. Furthermore, it is unclear whether these small, dense LDL particles are an independent risk factor for CHD or just reflect the general abnormalities associated with the dyslipidemia of NIDDM. LDL particle size was not independently associated with NIDDM in the study by Lahdenpera et al (1996). The concept of a threshold effect for TAG in determining LDL size was introduced by Lahedenpera et al (1996). Below the TAG threshold concentration of 1.7 mmol/L, other factors may have greater affect on LDL particle size (e.g. genetics). Above the TAG threshold concentration of 1.7 mmol/L, the prevalence of atherogenic small, dense LDL particles increases and serum TAG has a major effect on LDL particle size. Further research supports this finding (Austin et al, 1990; Griffin et al, 1994; Pometta and James, 1993). Therefore, for those individuals with NIDDM, the questions of whether target levels of TAG remain too high and how vigorous the treatment of mild hypertriglyceridemia should be in NIDDM require answers.

(I) LDL Particle Size and Hypertriglyceridemia in NIDDM

Hypertriglyceridemic NIDDM individuals secrete increased amounts of large, TAG-rich VLDL particles from which LDL is formed via VLDL delipidation. It has been found that in this situation, LDL apoB is cleared more slowly from circulation.

Hence, the VLDL and LDL residence time is increased thus, providing increased time for the formation of small, dense LDL (Packard et al, 1995; Taskinen et al, 1990). It has been found that the catabolism of small, dense LDL particles is slower than for normal particles. Hence, these particles have more time to be oxidized and also more time to penetrate the arterial wall (Nigon et al, 1991). Hypertriglyceridemic LDL have been shown to be taken up less efficiently by skin fibroblasts and the oxidation of hypertriglyceridemic LDL is increased. These changes enhance the atherogenicity of the LDL particle (Chait et al, 1993; McKeone et al, 1993). The influence of NIDDM on LDL particle size is mediated by serum TAG concentration and is not an independent regulator of LDL particle size. Increased levels of small, dense LDL particles appear at serum TAG concentrations in the upper normal range (Lahdenpera et al, 1996). Thus, treatment of mild hypertriglyceridemia in NIDDM patients is important in the prevention of its atherogenic effects.

(3) Lipoprotein Size and Coronary Heart Disease

Campos et al (1991a) found that smoking, hypertension, NIDDM, HDL-C, and LDL-C levels were strong discriminators between CHD and control subjects, while TAG and LDL particle size did not add significant information to their study model. Hence, small LDL particle size was determined not to be an independent risk factor for CHD after conventional risk factors and lipoprotein parameters (LDL-C and HDL-C) had been considered (Campos et al, 1991a).

The diabetic state is associated with subtle, qualitative changes in lipoprotein particle composition as well as quantitative effects. In addition to alterations in the absolute levels of lipids and lipoproteins, there is evidence that the composition of lipoproteins may be changed in the diabetic state. The level of small, dense LDL is increased in NIDDM but whether NIDDM is an independent determinant of LDL size is unclear (Lahdenpera, 1996; Reaven et al, 1993a). The significance of these changes remain to be determined and whether small, dense LDL is an independent risk factor for CHD or reflects other lipid abnormalities is unclear. NIDDM is associated with altered LDL lipid composition, especially LDL particle enrichment with TAG, producing LDL compositional changes (Uusitupa et al, 1993). Increased LDL-triglyceride concentrations have been described (Lahdenpera et al, 1996) as being related to increased VLDL-triglyceride concentrations which lead to increased LDL particle size (Lewis et al, 1972; Taskinen et al, 1986). These changes in LDL are important factors in determining its characteristics (Barakat et al, 1990; Coresh et al, 1993). In the multivariate regression analysis of Lahdenpera et al (1996), neither NIDDM nor CHD were associated with LDL particle size. However, serum TAG was the major determinant of LDL size in both NIDDM and non-diabetic subjects. Overall, in NIDDM subjects with or without CHD, serum TAG has been found to be the major determinant of the properties of LDL particles, including LDL particle size. For NIDDM individuals, the clinical implication of this finding is that serum TAG should be as low as possible to prevent atherogenic changes in LDL.

(4) LDL Oxidation

Increased lipid peroxidation has been commonly observed in NIDDM subjects (Ceriello et al, 1991; Niskanen et al, 1995). Babiy et al (1992) have shown that LDL from hypercholesterolemic NIDDM subjects is more easily oxidized. Similar findings in normocholesterolemic NIDDM have also been reported (Bowie et al, 1993). Free radical production (superoxide anion, O_2^-; hydrogen peroxide, H_2O_2; hydroxyl radical, OH) is increased in NIDDM suggesting an increased potential of LDL oxidation in NIDDM individuals (Ceriello et al, 1993; Kaji et al, 1985; Mullarkey et al, 1990). Excess free radicals in the vascular system can induce endothelial dysfunction and plaque formation (Kashiwagi et al, 1996).

LDL oxidation involves peroxidation of its PUFA (Esterbauer et al, 1987; Lenz et al, 1990; Reaven et al, 1991; Steinbrecher, 1987). LDL particles rich in PUFA are more readily modified and hence more atherogenic. However, PUFA enriched diets are considered beneficial due to their hypocholesterolemic effects (Mattson and Grundy, 1985). Therefore a PUFA diet may have two opposing effects but an overall favorable balance, i.e. antiatherogenic (Reaven et al, 1991). Because MUFA have been shown to be as effective as PUFA in lowering LDL-C levels, when they replace saturated fatty acids (SFA), use of MUFA in the diet rather than PUFA may confer additional protection to LDL by generating particles which are more resistant to oxidative modification (Reaven et al, 1991).

In vitro oxidation of LDL by copper can be divided into three phases or indexes: the lag phase (no diene production), the propagation phase (rapid increase in diene

production), and the decomposition phase where diene production reaches maximum (Kleinveld et al, 1993). The lag phase is considered a measure of susceptibility of LDL to oxidation. Factors considered to be responsible for LDL susceptibility to oxidation are believed to include the particles antioxidant content as well as its fatty acid composition. Linear correlations between the length of the lag phase and the major antioxidant content of the LDL particle, α- tocopherol, have not been found. From the kinetic absorbance profile during the oxidation of LDL, certain indexes can be determined which describe the oxidative stability of that particular LDL sample. The first index is the initial absorbance at 234 nm. The second index is lag time. However, because many factors determine this parameter, it is difficult to interpret its significance as related to LDL oxidation. The maximal rate of LDL oxidation is the third index used to evaluate oxidative stability of the LDL particle and is calculated from the slope of the absorbance curve during the propagation phase (expressed as nanomoles of conjugated dienes produced per minute per milligram LDL protein). The fourth index is the maximal amount of conjugated dienes formed. The total amount of conjugated dienes produced can be calculated by subtracting the initial absorbance at 234 nm from the final absorbance of the fourth index. The time, in minutes, required to reach maximal absorbance is the fifth index used to describe LDL oxidative stability and is called $t_{(max)}$. The formation of conjugated dienes is an early step in lipid peroxidation and is dependent on the fatty acid composition and endogenous antioxidants (mainly vitamin E) of the LDL particle (Witztum, 1991).

(i) Lipoprotein Interactions with Monocyte/Macrophages

Experimental animal models of atherosclerosis have implicated the monocyte as playing a central role in the formation of the early arterial lesion or fatty streaks (Faggiotto and Ross, 1984; Faggiotto et al, 1984; Joris et al, 1983). In later stages, the monocyte takes on macrophage characteristics and accumulates lipid to become the foam cells of the fatty streak (Betteridge, 1989). These lipid-rich macrophages appear to be toxic to the arterial endothelium which becomes disrupted allowing for platelet adhesion and aggregation, smooth muscle cell proliferation and progression of the lesion to a fibrous plaque (Faggiotto and Ross, 1984; Faggiotto et al, 1984). Modified LDL was shown to be rapidly taken up by macrophages through a receptor-mediated scavenger pathway process (Goldstein et al, 1979). The common feature of modified LDL, thought to be responsible for its interaction with the macrophage scavenger, is the presence of negatively charged residues (Goldstein et al, 1979). The specific modification of LDL required for *in vivo* macrophage transformation to foam cells remains to be determined. However, the early stage of the atherosclerotic lesion involves formation of oxidized LDL. It has been found that oxidized rather than native LDL delivers cholesterol to the macrophage (Sparrow et al, 1989), which is the precursor of the foam cell and the major cholesterol-containing cell in the atherosclerotic plaque. Several ways by which LDL oxidative modification can contribute to development of atherosclerosis have been suggested, including: (1) increased uptake of oxidized LDL (oxLDL) via macrophage scavenger receptor pathway (Steinbrecher et al, 1984); (2) chemoattractant activity of oxLDL leading to recruitment of additional monocytes to the affected area (Quinn et al,

1987); (3) oxLDL inhibition of macrophage motility leading to macrophage retention in the arterial intima (Quinn et al, 1987); and (4) oxLDL causing direct cellular injury (Morel et al, 1983). The effects of the NIDDM disease state on LP interactions with the scavenger receptor and LDL receptor on monocyte/macrophage cells remain to be fully determined. Whether oxidatively modified LDL is enhanced in NIDDM leading to increased interaction with the scavenger receptor also remains to be determined. However, lipid peroxides have been found to be increased in NIDDM subjects (Nishigaki et al, 1981). Increased superoxide formation by mononuclear cells from NIDDM patients has been described (Hiramatsu and Arimori, 1988). The effects of insulin on the classical LDL receptor in monocytes/macrophages have not been fully elucidated. No effect of insulin has been found in human monocyte/macrophages (Fogelman et al, 1981). However, evidence exists showing increased interaction of LP from NIDDM subjects with the LDL receptor; i.e. VLDL from NIDDM subjects with and without hypertriglyceridemia was taken up and degraded to a greater extent by mouse peritoneal monocyte/macrophages than normal VLDL (Kraemer et al, 1985).

(ii) The Relationship Between LDL Fatty Acid Composition and LDL Oxidation in NIDDM

The fatty acid composition of LDL may effect its susceptibility to oxidative modification. It is important to investigate the fatty acid composition of LDL as a potential risk factor contributing to the increased CHD seen in NIDDM. The fatty acid content of LDL and its relationship to LDL oxidizability was assessed by Dimitriadis et

al (1995). Normolipidemic NIDDM subjects had increased LDL susceptibility to oxidation suggesting that LDL fatty acid composition may contribute to this increase in oxidizability (Dimitriadis et al, 1995). The total quantity of linoleic acid (LA) in the LDL and the amount of LA in the cholesteryl ester fraction of LDL was significantly higher in NIDDM subjects when compared to normal subjects (Dimitriadis et al, 1995). In addition, the oxidizability of LDL was increased in the NIDDM group which was demonstrated by an increase in total conjugated diene formation. This indicates that the LDL of NIDDM patients is more susceptible to oxidative modification and hence, could be a possible factor affecting increased atherogenic risk via increased foam cell formation and acceleration of atheromatic plaque formation (Dimitriadis et al, 1995). Oxidation of LDL involves peroxidation of its PUFA (Esterbauer et al, 1987; Lenz et al, 1990; Steinbrecher, 1987). Some studies investigating the fatty acid composition of plasma lipids and LDL lipids in NIDDM patients have found lower levels of LA and higher levels of the more highly unsaturated LA metabolites including arachidonic acid (Pelikanova et al, 1991; Salomma et al, 1990). Others studies of NIDDM patients have demonstrated differing levels of LA metabolites along with similar plasma LA levels when compared to controls (Takahashi et al, 1984; Tivlis et al, 1988; VanDoomaal et al, 1988). Very few studies have examined the fatty acid composition of LDL in NIDDM subjects and none have identified gender differences in this parameter.

LDL core fatty acid composition is important to examine in NIDDM individuals, whether or not they are reasonably controlled, due to the possibility of enhanced LDL

oxidizability and its implications for foam cell and plaque formation (Dimitriadis et al, 1995). The increased risk of atherosclerotic disease in NIDDM individuals may be due to enhanced foam cell formation following an increased susceptibility of LDL to oxidative modification. LDL fatty acid composition may play a significant role in LDL oxidizability. Dimitriadis et al (1995) found the LDL of NIDDM subjects to have higher LA levels than controls and LDL oxidizability was also increased in the NIDDM group. Because the LDL from the NIDDM subjects was more susceptible to oxidation, this could accelerate *in vivo* foam cell formation resulting in increased atherosclerotic risk in the NIDDM state (Dimitriadis et al, 1995). The ratio of PUFA to SFA content of LDL has been suggested to contribute to its oxidative susceptibility (Esterbauer et al, 1992; Reaven et al, 1991). Kleinveld et al (1993) found that, in normal subjects, LDL rich in OA and poor in LA was less easily oxidized and that LDL susceptibility to oxidation is partly determined by its fatty acid composition, particularly by its ratio of OA content to LA content. Reaven et al (1991) reported on the feasibility of altering the diet in a way that would not raise LDL-C and yet would decrease the susceptibility of LDL to oxidative modification. Also, a study by Laitinen et al (1993) showed that the serum fatty acid composition of NIDDM individuals could be changed through diet therapy.

The study by Dimitriadis et al (1995) is the only one that has been found which examines the fatty acid composition of LDL isolated from NIDDM subjects. This area of study is important to explore because of the possible relationship between LDL composition and its susceptibility to oxidation. The increased susceptibility of LDL from NIDDM individuals to oxidation compared to the non-diabetic population suggests the

LDL fatty acid composition may be a contributing factor in the increased LDL oxidizability in NIDDM and hence, increased development of atherosclerosis. When the relationship between LDL fatty acid composition and its oxidizability was examined by Dimitriadis et al (1995), there was a significant, positive correlation between the percentage of LA in the LDL cholesteryl ester fraction and the maximum amount of conjugated dienes produced. The reason NIDDM have increased LA in the cholesteryl ester fraction of LDL is unknown. However, it may be due to diet as dietary recommendations for NIDDM is to decrease SFA and to replace it with more unsaturated sources of dietary fat, which have been shown to increase serum LA (Parfitt et al, 1993). Increased LA in LDL has been suggested to result from an impairment in the activity of the insulin sensitive Δ-6 desaturase in NIDDM (Mooradian, 1991). The extent of LDL oxidation was shown to be strongly influenced by the LDL LA content by Reaven et al (1993b). Other dietary studies show that the enrichment of LDL LA by dietary manipulation results in an increase in LDL oxidizability (Bonanome et al, 1992; Reaven et al, 1993b).

(5) LDL and Atherosclerosis

LDL is the major cholesterol-carrying LP and is characterized as the most atherogenic due to the direct relationship between LDL-C concentration and risk of CHD. Over 60% of LDL clearance from plasma is mediated through binding to the LDL receptor and its endocytosis; most occurring as a result of ligand-receptor binding within the liver. Evidence suggests that in the artery wall, the LDL receptor is not a major factor

in the handling of LDL reaching the intima. Chemically modified LDL is taken up by monocytes in cell culture and is believed to be due to the existence of other receptors called scavenger receptors on macrophages. These receptors recognize LDL which has been modified either by acetylation or conjugation with malondialdehyde, an aldehyde product of lipid peroxidation. Modified LDL loses its ability to be recognized by the LDL receptor and is a strong chemoattractant for human monocytes due to alterations in the lipid moiety of the LDL (accumulation of significant amounts of lysophosphotidylcholine).

(i) Nature of LDL Modification

Changes occurring in the LDL molecule depend on peroxidation of PUFA in the lipid fraction and therefore on free radical generation (Woolf, 1990). Modification involves changes in lipid and protein moieties. In the lipid there is a significant degree of conversion of lecithin (phosphotidylcholine; PC) to lysophosphotidylcholine; the cytotoxic effects of modified LDL on endothelial cells in culture probably depends largely on this conversion (Cathcart et al, 1985; Henrikson et al, 1979). In the course of peroxidation of unsaturated lipid moieties, 4-hydroxyalkenals can be generated from n-6 and n-3 PUFA. These biologically active compounds have been found to have cytotoxic effects (Jurgens et al, 1987) and can cause severe disturbances of cell function at a number of levels (Estebauer et al, 1988a).

Steinbrecher (1987) has shown that cleavage products of arachidonic acid can react with apoB and this reaction involves blocking of the epsilon-amino groups of lysine

residues. It is believed that the interactions of aldehydic lipid peroxidation products with the epsilon-amino groups of lysine produces new epitopes which are recognized by the scavenger receptor on macrophages (Estebauer et al, 1988b). Foam cell formation may be mediated through the operation of the scavenger receptors and monocytes may be attracted to the relevant area of the intima and immobilized there.

(ii) Oxidatively Modified LDL and its Role in Atherosclerosis

Post-secretory atherogenic modifications of LDL, namely, peroxidation of LDL lipids elicits a cascade of chemical changes which initiate a complex cluster of biological responses from a number of different types of target cells. Modified LDL becomes a strong chemoattractant for monocytes (Woolf, 1990) and this effect may be a primary event in the initiation of the fatty streak (Steinberg, 1988; Steinberg et al, 1989). Oxidatively modified LDL (oxLDL) may represent one of the more potent atherogenic manifestations of LDL (Dimitriadis et al, 1995). However, oxidized LDL is not a single homogeneous entity and depending on the nature of the oxidation product, the extent of oxidation, and the accompanying changes, a wide range of oxidized LDL particles may be generated (Parthasarathy, 1991). Oxidation of LDL leads to its enhanced uptake by macrophages which is believed to result in foam cell formation and atherosclerosis (Steinberg et al, 1989; Witztum and Steinberg, 1991). Research has shown lipid peroxidation is intimately tied to LDL modification (Parthasarathy, 1991; Steinberg et al, 1989). Other cell types capable of oxidizing LDL include smooth muscle cells, fibroblast, monocytes, and macrophages. The cell-induced modification can be

mimicked by the addition of copper to LDL in simple buffers (Kleinveld et al, 1993; Steinbrecher et al, 1984). The uptake and degradation of the oxLDL by the macrophages is specific and saturable and is not competed for by native LDL. Recognition of the oxLDL by macrophages rests with the protein moiety of the oxLDL.

(iii) Changes in LDL During Oxidative Modification

Originally, the term 'modification' was used to describe modifications of apoB that lead to altered receptor recognition. However, oxidation of LDL lipids and oxidative modification of LDL may be two separate aspects of the oxidative process. This is the distinction between the events that initiate peroxidation of LDL lipids and apolipoprotein modification. Researchers have established lipid peroxidation as an important event in the oxidative modification of LDL (Esterbauer et al, 1987; Quehenberger et al, 1987). These studies showed that during the initial stages of LDL oxidation, rapid depletion of LDL-associated antioxidants (most importantly α-tocopherol) occurred. Following the consumption of the antioxidants, polyunsaturated fatty acids (PUFA) were oxidized. The PUFA undergo extensive decomposition which leads to formation of different aldehyde compounds each with potent biological properties. Some LDL phospholipids undergo hydrolysis at the 2-position as a result of LDL oxidation thus producing lysophosphotidylcholine. The removal of the oxidized fatty acids from the particle surface has been suggested to aid in the rapid propagation of lipid peroxidation into the core of the LDL particle (Parthasarathy, 1991).

Oxidation of LDL lipids also affects the apoB protein moiety of the lipoprotein. It is most likely that lipids of the core, upon oxidation, contribute significantly to the generation of the modified apoprotein (Parthasarathy, 1991).

The cholesterol moiety of LDL is oxidized as well. Several oxy-sterols have been identified in oxLDL. The hydroperoxy derivatives of cholesterol appear to have potent biological properties and may be responsible for the cytotoxicity of oxLDL (Jialal et al, 1991; Zhang et al, 1990).

(iv) Mechanisms of LDL Oxidation

Each type of cell that has the ability to oxidize LDL may utilize different mechanisms to induce the oxidation process. It has been suggested that cells oxidize LDL by three main methods. The first process is by generating reactive oxygen species such as superoxide radicals (Cathcart et al, 1989; Heinecke, 1986), the second is through the use of specific fatty acid oxygenases (Parthasarathy et al, 1989; Rankin et al, 1991), and the third involves secreting substances that provide oxidants into the extracellular medium (Heinecke et al, 1987). The exclusive involvement of superoxide anions in the oxidative modification of LDL by endothelial cells appears unlikely, although it may play a role in LDL oxidation by smooth muscle cells or monocytes.

Several recent findings suggest that cellular lipoxygenases, particularly 15-lipoxygenase activity, may be involved in LDL modification by some cell types (Parthasarathy et al, 1989; Rankin et al, 1991; Sparrow et al, 1988; Yla-Herttula et al,

1990; Simon et al, 1989). The mechanisms by which lipoxygenases may contribute to the oxidation of LDL is not known.

(v) The Biological Effects of Oxidized LDL

Identified atherogenic effects of oxLDL include (1) degradation of oxLDL faster than native LDL by macrophages, (2) serving as a chemotactic agent for monocytes, (3) inhibition of macrophage chemotaxis, (4) being cytotoxic to cells, (5) inhibition of endothelium-dependent relaxation, (6) enhancement of monocyte adhesion to endothelial cells, (7) induction of endothelial cell expression of granulocyte and macrophage colony stimulating factor, and (8) stimulation of macrophage chemotactic peptide-1 from endothelial cells and smooth muscle cells (Berliner et al, 1989; Cushing et al, 1990, Rajavashisht, 1990).

Several research groups have shown evidence which indicates the presence of oxidatively modified LDL *in vivo* (Bjorkhem et al, 1991; Hoff and O'Neil, 1991; Yla-Hettuala et al, 1989). Most notably, (1) antioxidants (probucol) prevent the development and progression of atherosclerotic lesions in WHHL rabbits (Carew et al, 1987; Kita et al, 1987), probucol specifically inhibits the degradation of LDL in macrophage foam cells, (2) autoantibodies directed towards oxLDL are present in human plasma, (3) LDL isolated from atherosclerotic lesions has some of the characteristics of oxLDL (Yla-Herttula et al, 1989; Hoff and O'Neil, 1991), and (4) lysine-aldehyde adducts, which may indicate the presence of oxLDL, can be demonstrated in macrophage-rich lesions of human aorta (Palinski et al, 1989).

OxLDL is rapidly removed from the circulation by the liver and its clearance is directly related to its extent of oxidation (Steinberg et al, 1989). However, Berliner and co-workers (Berliner et al, 1989; Cushing et al, 1990; Rajavashisht, 1990) have described a minimally oxLDL which is not sufficiently modified as to be recognized by the scavenger receptor and therefore may have a longer half-life in the plasma. When this particle enters the arterial wall, it may suffer the fate of further oxidative degradation. The toxicity of diabetic plasma has been attributed to lipid peroxidation products. LDL fractions from streptozotocin-induced diabetic rat plasma contained higher levels of peroxides and were toxic to cultured cells (Morel and Chisolm, 1989).

III The Pathology of Atherosclerosis

The pathogenesis of atherosclerosis has been described by many researchers (Hornstra, 1989; Leaf and Weber, 1989; Ross, 1986; Woolf, 1990; Zeman, 1983). Figure 2 outlines the pathogenesis of atherosclerosis (Mullin, 1993). Atherosclerosis is defined as a dynamic state involving four principle cell types (endothelium, smooth muscle, platelets, and monocyte/macrophages) which contain or can synthesize and release chemoattractants and growth factors (one of which is platelet derived growth factor; PDGF). Atherosclerosis is characterized by three interacting processes (Woolf, 1990). The first of these processes involves accumulation and modification of plasma derived lipid within the arterial intima leading to the accumulation of lipid-filled macrophages within the affected intima. This step is thought to be mediated by the oxidative modification of LDL-C leading to foam cell formation and hence, fatty streaks

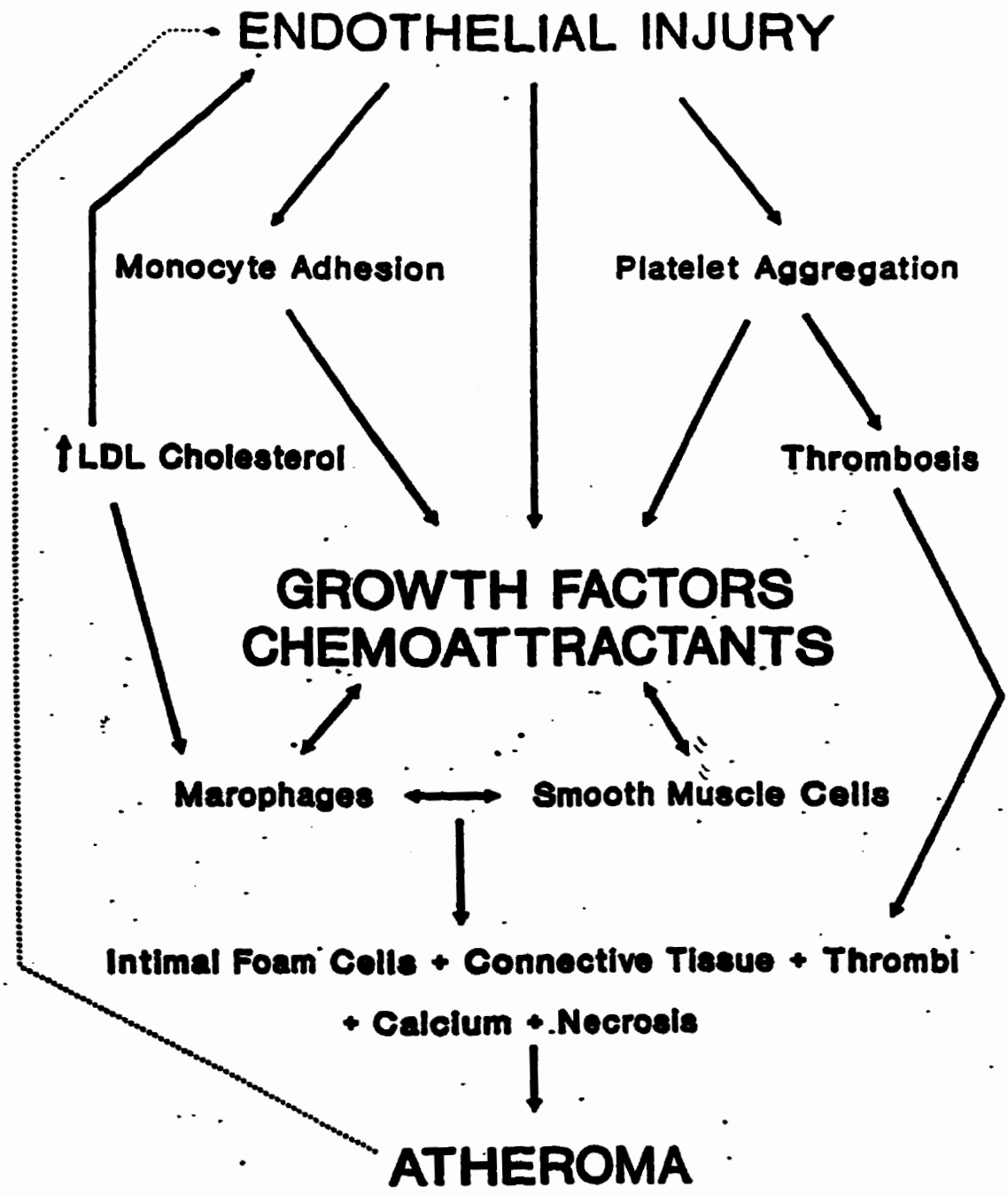


Figure 1. Pathogenesis of Atherosclerosis (From C.R. Mullin, 1993)

which are the earliest lesion of atherosclerosis (Ross, 1986); a majority of foam cells in fatty streaks are macrophages. Secondly, connective tissue proliferation occurs due to alterations in the functional phenotype of the intimal smooth muscle cells (SMC) as a result of interactions with growth factors including platelet derived growth factor (PDGF). SMC proliferation is a key event in determining the extent of plaque formation (Ross, 1986). SMC contain LDL growth factors and receptors and are able to respond to several chemotactic factors. The capacity of SMC to migrate in response to these factors plays an important role in their ability to accumulate in the atherosclerotic lesion (Ross, 1986). PDGF is a mitogen (Kohler and Lipton, 1974; Ross et al, 1974) and chemoattractant (Deuel et al, 1981; Grotendorst et al, 1982) responsible for migration and proliferation of SMC from the arterial media and is seen in atherogenesis and arterial injury. PDGF is stored and released from the α-granules of platelets but can also be produced by endothelial cells, macrophages, and arterial smooth muscle cells in their synthetic phenotype. Platelets from NIDDM subjects have been shown to exhibit abnormal behaviors including increased adhesion, aggregation, and thomboxane production (Ishii et al, 1992). These abnormalities are considered to be involved in the pathogenesis of diabetic vascular disease. Platelet hyperfunction could lead to enhanced PDGF release from activated platelets at the local vessel wall sites. It is unclear whether the alterations in platelet function of NIDDM is a direct effect of hyperglycemia or due to other metabolic disorders present in the disease. Modulation of arterial smooth muscle cells from contractile to the synthetic phenotype is responsible for connective tissue proliferation which is the key element in the evolution of atherosclerosis. Proliferation of

connective tissue is a platelet-driven response resulting in formation of a sub-endothelial fibromuscular cap which may compromise the integrity of the arterial lumen. Finally, connective tissue necrosis forms a soft, deformable atheromatous pool at the plaque base. This process is less well understood and is thought to involve activated macrophages and is important as a risk factor for acute thrombosis. Atheromatic plaques consist partly of a lipid rich pool of necrotic connective tissue at the plaque base and partly of a fibromuscular cap on the lumenal aspect of the atheromatus pool.

(a) Stages of Atherosclerosis

One of the first stages of atheroma formation is adhesion of monocytes to the endothelial surface, entry into subendothelial tissues and ingestion of large amounts of lipid with ultimate conversion to foam cells and development of fatty streaks (Rosenfeld et al, 1987). Monocyte traffic between the blood and artery wall occurs. The fatty streak cell population shows a significant proportion of cells originating from monocytes as well as showing macrophage markers. Recognition that the macrophage forms a significant element in the cell population of fatty streaks and of mature atherosclerotic lesions is an important advance toward further understanding the mechanisms involved in atherogenesis. The macrophages may exercise a range of functions within the arterial intima which greatly transcend their conventional role as a phagocytic cell.

A combination of morphological and epidemiological data suggest that some fatty streaks originating early in life undergo regression while others progress to mature atheromatic plaques. The latter course of events is more likely to occur in those

populations whose degree of exposure to risk factors for atherogenesis such as hyperlipidemia, high blood pressure, and diabetes mellitus is great. The extent of intimal involvement by the fibrolipid plaque appears to predict the frequency and severity of the clinical manifestations of atherosclerosis in given population groups. All fibrolipid plaques share two basic morphological components: (1) a connective tissue cap lying beneath the endothelium and (2) an underlying atheromatous pool of lipid rich, largely necrotic, debris. Fibrolipid plaques are associated with three changes in the surrounding endothelial tissue: (1) increased fibrous tissue, (2) increased vascularity, and (3) frequent presence of cellular aggregates consisting mostly of β-lymphocytes. Lymphatic infiltrates are the expression of an immune response to the presence of oxidized lipids and ceroid in intralesional macrophages.

The fatty streak is characterized by the build up of intra- and extra- cellular lipids derived from LDL (Parthasarathy, 1991). Recent evidence suggests macrophages are the predominant cellular origin of these cells. The basic mechanism(s) by which cells take up LDL begins with the internalization of the LDL molecule by cells with a specific receptor that recognizes the apolipoprotein B_{100} (apoB) on the particle. The LDL receptor is down-regulated when cellular requirements for cholesterol is met suggesting that excessive accumulation of lipids by arterial foam cells is not mediated by the LDL receptors (Hobbs et al, 1989). Receptor(s) other than the LDL receptor must be involved in the uptake and accumulation of lipids by macrophages. The inability of native LDL to generate foam cells with macrophages led Brown et al (1980) to propose that some form of post-secondary modification of the LDL may be involved which resulted in modified

LDL being susceptible to enhanced uptake and degradation by the cell. Subsequently, it was discovered that acetyl LDL, in which the ε-amino groups of the lysine residues are chemically acetylated, generate a negative charge on the amino group making them readily degradable by macrophages (Brown et al, 1980). Over 70% derivatization of the amino groups of the lysine residues is required for recognition of the altered lipoprotein by the cells. The degradation of acetyl LDL by macrophages is mediated by a saturable high affinity receptor referred to as the scavenger receptor or the acetyl-LDL receptor. Native LDL is not recognized by this receptor. The scavenger receptor is localized predominantly in the macrophage-rich areas of the atherosclerotic lesion. Whether this is the only receptor involved in the uptake of modified lipoproteins or one of a group of scavenger receptors is not known. However other receptors recognizing distinct forms of modified LDL have been suggested (Sparrow et al, 1989). It is unlikely that extensive acetylation of the lysine residues of apoB will occur in the body. However, the concept that negatively charged lipoproteins may interact with macrophages lead to intensive research for other more biological modifications. Fogelman et al (1980) reported that malondialdehyde (MDA), known to be generated from aggregated platelets or from lipid peroxidation, could covalently modify LDL lysine residues and produce a negatively charged LDL capable of interacting with macrophages. As well, Steinberg et al (1989) observed that LDL, when incubated with certain cells, acquired a negative charge and was avidly degraded by macrophages. This was referred to as endothelial cell-modified LDL. Extensive study of this model has subsequently shown that lipid peroxidation was the key determinant factor in the modification of LDL by cells (Steinbrecher et al, 1989).

All these modifications of LDL which ultimately lead to an altered charge on the lipoprotein by derivitizing the ε- amino residues of the lysine, differ vastly in their ability to convert LDL to a form recognized by the scavenger receptor. The modification of ε-amino groups of the lysine residues of the apoB has been the primary focus of research. However, the ability with which the receptor interacts with other polyanionic compounds suggests other potential sites of modification. In fact, the domain of the LDL, whose changes in structure or configuration lead to scavenger receptor recognition, has yet to be determined.

IV Dietary Treatment Approaches in the Management of NIDDM Individuals

Nutrition counseling for the NIDDM patient is of prime importance as it aids in the continual management of the disease and also addresses treatment of the common secondary factors associated with CHO and lipid metabolism dysfunction, most importantly obesity. It has been suggested that the most important dietary modification is a reduction in total fat content to approximately 30% of daily energy of which 10% is PUFA, 10% is MUFA and 10% is SFA in a low-fat, high-CHO diet. The main goal of this diet is to decrease CHD risk by reducing TC and LDL-C and by favoring weight loss in obese NIDDM individuals. Weight loss in obese NIDDM individuals is very important as it results in dramatic improvements in glucose tolerance and lipid metabolism. The type of dietary fat that is recommended is becoming increasingly recognized as playing an important role in diet modification. Advantages of MUFA over PUFA are becoming apparent as MUFA seem to be as effective as PUFA as a substitute

for saturated fat with respect to lowering plasma lipids and may be advantageous as plasma HDL-C levels are unaffected by MUFA (McDonald et al, 1989).

A considerable number of NIDDM patients are in poor glycemic control which is often associated with obesity. These patients also tend towards consistently low plasma HDL-C, elevated triglycerides, and sometimes elevated plasma cholesterol levels (Coulston et al, 1989).

Extensive evidence exists to demonstrate that low-fat, high-CHO diets lead to increased plasma triglyceride concentrations (Coulston et al, 1987; Coulston et al, 1989; Hollenbeck et al, 1984; Grundy, 1988; Liu et al, 1983; Sestoft et al, 1985; Garg et al, 1988; Rivellese et al, 1990; Riccardi et al, 1984). Therefore, the use of a low-fat, high-CHO diet for NIDDM patients may be seen as a disadvantage as hypertriglyceridemia is a likely consequence if these individuals adhere to the current dietary recommendations to increase dietary CHO. Another deleterious effect attributed to low-fat, high-CHO diets in NIDDM patients is a significant decrease in HDL-C concentrations (Coulston et al, 1987; Coulston et al, 1989; Sestoft et al, 1985; Garg et al, 1988; Rivellese et al, 1990). HDL-C levels are inversely associated with the development of CHD and because HDL-C levels are known to be at lower than 'normal' levels in NIDDM patients, any adverse effects of the low-fat, high-CHO diet cannot be ignored (Beach et al, 1979; Miller and Miller, 1975; Reckless et al, 1978). Deterioration of CHO metabolism is also an observed effect after increasing dietary CHO in NIDDM individuals. Increasing dietary CHO resulted in increased day-long glucose and insulin concentrations (Coulston et al, 1987; Coulston et al, 1989; Sestoft et al, 1985; Garg et al, 1988; Rivellese et al, 1990).

The only known study which measured insulin action in NIDDM patients reported no significant improvement in insulin action after these subjects consumed diets containing 60% daily energy as CHO (Garg et al, 1989). Substantial evidence suggests that diets containing conventional quantities of fat (~40% of daily energy), in which the composition of fat has been modified to reduce SFA and dietary cholesterol levels, appear to offer the most beneficial control of CHO and lipoprotein metabolism in NIDDM (Coulston et al, 1989; Hollenbeck and Coulston, 1991).

Data from many studies support the importance of the type of dietary fat in regulating lipoprotein metabolism of NIDDM individuals. Replacement of saturated fat with unsaturated fat in the context of the conventional diet seems to produce the desired reduction in plasma total and LDL-cholesterol levels and avoids the exacerbation of hyperglycemia, hyperinsulinemia, hypertriglyceridemia, and reduced HDL-C concentrations.

However, the effect of dietary alterations in CHO and fat content on plasma lipid and lipoprotein levels, glucose tolerance, and insulin sensitivity is a controversial issue. Weight reduction remains a cornerstone of dietary treatment in obese NIDDM patients as dramatic improvements in glucose tolerance are seen in those who lose weight; obesity also has detrimental effects on CHO and lipid metabolism in NIDDM patients. Lipid and lipoprotein metabolism dysfunction and dietary fat intake are closely related to the development of atherosclerosis, NIDDM, and obesity. Treatment of the CHO intolerance and dyslipidemia found in NIDDM is a primary goal of diet therapy. However, the effect that differences in diet composition have on the metabolic dysfunctions of the NIDDM

syndrome are not clearly understood. The impact that specific dietary changes have on the metabolic risk factors for CHD known to exist in NIDDM individuals requires further study.

(a) Effect of MUFA and PUFA Diets on Lipoprotein Metabolism and NIDDM

Well established recommendations for dietary treatment are to reduce SFA intake as this type of fat is known to elevate blood TC (Hegsted et al, 1965; Keys et al, 1957). However, there is considerable debate over what should replace SFA. Previous recommendations were that a high-CHO diet should be employed (Howard et al, 1991). However, due to concerns about adverse effects of this diet, unsaturated fatty acids have become more popular as a choice to replace SFA. The question then becomes what type of unsaturated fatty acids, MUFA or PUFA?

Initially, Keys et al (1965) suggested that MUFA played a neutral role in lipoprotein metabolism and that PUFA were more effective than MUFA or CHO at lowering plasma TC and LDL-C. However, MUFA were found to be equally as effective at lowering TC and LDL-C as PUFA by Mattson and Grundy (1985) and Mensink and Katan (1989).

Canola oils' characteristic low level of saturated fatty acids (≈ 6%) along with high levels of MUFA (≈ 58%) and moderate PUFA levels (≈ 26% LA and ≈ 10 % LNA) are major advantages to its nutritional properties and value. Several studies have confirmed findings that canola oil is as effective (Wahrburg et al, 1992), and in some cases more effective (Lichtenstein et al, 1993), than fats rich in PUFA (such as sunflower

and soybean oils) in decreasing plasma total cholesterol (mainly LDL-C) in normolipidemic individuals (Wardlaw et al, 1991; McDonald et al, 1989; Chan et al, 1991; Valsta et al, 1992). However, the nutritional benefit of canola oil regarding its content of LNA and/or its balance of PUFA (18:2/18:3 ratio) has not been clearly established. Chan et al (1991) found OA, LA, and LNA to be equally hypocholesterolemic, regarding TC and LDL-C levels, in non-diabetic male subjects and to have no adverse effects on HDL-C levels. Research has confirmed earlier studies which found MUFA equally as effective as PUFA in lowering plasma total and LDL cholesterol levels without negatively affecting plasma HDL levels (Mattson and Grundy, 1985; Mensink and Katan, 1989). Previous research had concluded PUFA lower HDL-C levels (Mattson and Grundy, 1985; Wahrburg et al, 1992; Lichtenstein et al, 1993), however, these studies commonly used unrealistically high PUFA levels in the experimental diets (28% total energy as PUFA compared to the regular level of 7%). When experimental diets contained more realistic PUFA levels, HDL-C levels were not affected (McDonald et al, 1989; Mensink and Katan, 1989; Chan et al, 1991; Wardlaw et al, 1991; Barr et al, 1992). Canola oil has also been found equally effective in lowering plasma TC and LDL-C levels on a low-fat (30% total energy) diet (Corner et al, 1990; Mata et al, 1992).

The premature atherosclerotic disease seen in NIDDM is closely related to dyslipidemia which includes increased levels of TC and LDL-C, decreased HDL-C, and increased TAG levels (Coulston et al, 1989). However, that NIDDM patients have

increased TC and LDL-C has been disputed with the contention that the main dyslipidemia is hypertriglyceridemia and decreased HDL-C levels (Ginsberg, 1996; Howard, 1987). Because NIDDM is a clinical disorder known to be altered by the level and type of dietary fat, current dietary therapy for these individuals aims at lowering the plasma lipids mentioned above and, in turn, the morbidity and mortality associated with CHD. Low-fat, high-CHO diets have been shown to be effective in lowering plasma TC and LDL-C in NIDDM but they tend to exacerbate the hypertriglyceridemia, decrease HDL levels even further (Betteridge, 1989; Coulston et al, 1987; Fontbonne, 1991; Sestoft et al, 1985) and increase plasma insulin levels and hyperglycemia (Garg et al, 1992a; Hollenbeck and Coulston, 1991). All of these factors lead to increased CHD risk and, for these reasons, the low-fat, high-CHO diet therapy has been challenged by Hollenbeck and Coulston (1991) and Grundy (1991) as being ineffective at reducing CHD in NIDDM. Both research groups have demonstrated that a high intake of MUFA, even at the more conventional dietary fat level of 40% total energy as fat, is more effective at normalizing blood lipid and insulin levels and glycemic control than low-fat, high-CHO diets. Similar findings have been reported by Parillo et al (1992) in that a reduction in consumption of complex CHO associated with an increase in consumption of MUFA improved glycemic control and peripheral insulin sensitivity in NIDDM subjects and the improvement was almost equal in magnitude to that achieved with insulin therapy or oral hypoglycemic agents (Andrews et al, 1984; Mandarino and Gerich, 1984). High MUFA intakes in NIDDM patients have been found to decrease postprandial plasma

glucose and insulin concentrations, decrease plasma TAG, and to have a negligible effect on TC and LDL-C which remain at normal levels (Garg et al, 1988; Grundy, 1991).

Studies with canola oil in normolipidemic, non-diabetic subjects (Chan et al, 1991; McDonald et al, 1989), as well as more recent reports that found MUFA improve glycemic control and plasma lipid patterns in diabetics (Hollenbeck and Coulston, 1991), suggest canola oil may prove beneficial in the treatment of NIDDM. The proposed alternate diet for NIDDM individuals responds to the characteristic defects in CHO and lipoprotein metabolism which are aggravated by the recommended low-fat, high-CHO diet. The main question this diet aims to address is: what is the optimal balance between dietary CHO and fat that will prevent deterioration of lipoprotein levels and peripheral insulin sensitivity? Overall, there is a similar decrease in plasma TC and LDL-C levels on both low or higher fat diets when SFA are replaced with either MUFA or PUFA (Barr et al, 1992). The next level of consideration when comparing the beneficial properties of MUFA with PUFA is their effect, if any, on the oxidative susceptibility of LDL-C. MUFA are less susceptible to chemical oxidation than PUFA. Hence, if a reduction in the amount of oxidatively modified LDL-C occurs, a reduction should occur in the involvement of these highly atherogenic lipoprotein particles in foam cell production and plaque formation. MUFA therefore, seem preferable to PUFA because PUFA may promote LDL oxidation, thus resulting in more atherogenic LDL (Grundy, 1991).

(b) Isocaloric Substitution of CHO for Fat in Normal Glucose Tolerant Individuals

A high saturated fat diet promotes deceased LDL receptor activity, increased TC and LDL-C levels, and increased CHD risk. When complex CHO replaces SFA in the diet, the inhibition of SFA on LDL receptors is removed, hence improved lipoprotein metabolism is favored, especially hepatic removal of LDL; the concomitant reduction of TC and LDL-C reduces CHD risk. In addition, weight reduction is favored, which is an added basis for the current recommendations for the low-fat, high-CHO diet.

Isocaloric substitution of CHO for fat in the diets of individuals with normal CHO and lipid metabolism have been found to produce significant increases in fasting total plasma triglyceride levels and reductions in HDL-C concentrations (Grundy, 1988; Betteridge, 1989; Fontbonne, 1991), both of which are independent risk factors for CHD. These deleterious effects on lipoprotein metabolism occurred without the expected improvements in total and LDL cholesterol concentrations. Also, postprandial triglyceride and insulin concentrations were found to be significantly elevated (Grundy, 1988) and fasting triglyceride concentration increased (Liu et al, 1983) after a low-fat diet. Overall, recent evidence suggests that low-fat, high-CHO diets do not provide any advantages in decreasing total plasma and LDL cholesterol concentrations over diets in which saturated fats were replaced with unsaturated fats, including MUFA or PUFA (Garg et al, 1992a). High-CHO diets tend to produce significantly higher fasting and post-prandial glucose and triglyceride concentrations, increased post-prandial insulin concentrations, and lower HDL-C levels (Mattson and Grundy, 1985; Mensink and Katan, 1989; Barr et al, 1992; Wahrburg, 1992), all of which are identified as being

associated with increased CHD risk. A decrease in HDL-C levels without a decrease in total or LDL cholesterol levels is clinically important as the ratio of total to HDL cholesterol has been associated with increased risk of atherogenesis; the higher the ratio value, the greater the CHD risk.

(c) Isocaloric Substitution of CHO for Fat in Non-Insulin-Dependent Diabetes Mellitus

Isocaloric substitution of CHO for fat in the diets of NIDDM patients results in similar effects as those seen in individuals with normal glucose tolerance. Given the pathology of the disease, the effects of a low-fat, high-CHO diet could be significantly more detrimental for NIDDM patients, in terms of increased risk of CHD. However, weight reduction in NIDDM is also a major consideration and may be facilitated by this diet. Walker and colleagues (1996) showed that equal amounts of body fat were lost by NIDDM subjects consuming a high-CHO, low-fat diet as those consuming a diet high in MUFA containing 35% fat. Many studies have indicated that substitution of CHO for fat results in increased postprandial plasma glucose and insulin levels, increased VLDL triglyceride concentrations, and reduced HDL-C concentrations without significantly affecting plasma total or LDL cholesterol concentrations (Coulston et al, 1987; Coulston et al, 1989; Garg et al, 1988; Garg et al, 1992a; Hollenbeck and Coulston, 1991; Parillo et al, 1992; Rivellese et al, 1990; Sestoft et al, 1985).

Research (Garg et al, 1988; Garg et al, 1992a; Garg et al, 1992b; Parillo et al, 1992) has shown that replacing saturated fat with MUFA results in significant metabolic

advantages over replacement with complex CHO. No significant changes in fasting plasma total or LDL cholesterol concentrations were found on diets in which total daily energy from fat was reduced from 50 to 25 %. Fasting total plasma triglyceride and VLDL concentration as well as the ratio of total to HDL cholesterol were significantly increased and HDL-C concentration decreased on the high-CHO, low-fat diet (Barr et al, 1992; Grundy, 1988; Mensink and Katan, 1989). Additionally, glycemic control deteriorated on the high-CHO, low-fat diet, as evidenced by significant increases in post-prandial plasma glucose, insulin, and triglyceride levels. Overall, these studies indicate that when CHO is substituted for saturated fat in the diet of NIDDM individuals, deleterious effects occur to CHO and lipoprotein metabolism which increases the risk of atherogenesis and CHD.

V Study Rational

Premature CHD is the most important cause of morbidity and mortality, for non-insulin-dependent (type II) diabetes mellitus (NIDDM) patients. Current dietary recommendations emphasize a low-fat, high-complex CHO diet to reduce plasma total and LDL cholesterol. However, evidence suggests this diet impairs glycemic control, exacerbates the hypertriglyceridemia found in NIDDM patients and decreases pre-existing low HDL-C levels. By contrast, high-fat diets rich in oleic acid (MUFA; C18:1) have been found effective in normalizing blood lipid and LP levels in NIDDM subjects. However, the effect of dietary oleic acid in a low-fat, high-CHO diet has not been established. Thus, canola oil which contains high levels of MUFA (oleic acid) and low

SFA levels, may benefit NIDDM patients when incorporated into a low-fat, high-CHO diet. In addition, MUFA are less easily oxidized than PUFA, thus canola oil may decrease the susceptibility of LDL to oxidation and hence, lower atherosclerotic plaque formation; oxidized LDL has been shown to be taken up by macrophages which become foam cells that initiate plaque formation in arterial vessel walls. Since canola oil has not been used in clinical trials with type II diabetics, an objective of the present study was to determine whether the oil offers any nutritional benefit in the treatment of NIDDM which is known to be altered by the level and type of dietary fat.

Plant breeders recently developed a low LNA canola cultivar which is appreciably more oxidatively stable than regular canola oil, offering distinct marketing advantages due to improved storage qualities. However, the question remains as to whether the relatively higher level of LNA in regular canola oil imparts nutritional benefit to the oil.

NIDDM patients exhibit a substantially greater incidence of CHD than the general population due to abnormal lipid patterns; therefore, it is possible that normolipidemic subjects are not sensitive enough to diet changes to show the small nutritional differences that may exist in canola oil versus low LNA canola oil, and that diabetes may offer a model for studying the possible nutritional benefits of LNA. Hence, a primary objective of the present study was to assess the nutritional properties of low LNA canola oil in the diets of NIDDM patients to determine whether LNA in canola oil imparts any nutritional benefit to the oil beyond those that might occur because of the high oleic acid, low SFA levels of canola oil. This study is the first, to our knowledge, specifically designed to

assess the nutritional properties of canola oil in the clinical treatment of NIDDM. Thus the null hypotheses tested in the study were:

(i) when compared to a regular low-fat diet (30% daily energy from fat), canola oil, low LNA canola oil, and sunflower oil offer no nutritional advantages in the clinical treatment of NIDDM;

(ii) the LNA content of regular canola oil does not impart it any nutritional benefit compared to the low LNA content of low LNA canola oil; i.e. the nutritional properties of regular and low LNA canola oil do not differ; and

(iii) regular canola oil, low LNA canola oil, and sunflower oil have similar and negligible effects on changes in the blood lipid parameters of NIDDM patients, including total cholesterol, LDL-C, HDL-C, and TAG concentrations as well as LDL oxidative susceptibility, fatty acid composition, and particle size

MATERIALS AND METHODS

I Experimental Design

The experimental model used was a 56 day randomized block design. Each subject was randomly assigned to one of four experimental diets: a regular canola oil diet (CAN), a low linolenic acid canola oil diet (LLNA), a sunflower oil diet (SNFLR), or a control diet (CONT; the subjects followed their usual dietary patterns). The subjects were free-living, non-insulin-dependent (type II) diabetics who were supplied with spread and oil corresponding to the assigned diet. The control subjects were not supplied with spread or oil. Subjects were counseled by the Diabetes Education Centre dietitians and a dietitian working on the study to maintain a low-fat (30% of total energy), high-complex carbohydrate diet in keeping with current diet therapy for type II diabetics. Twelve-hour fasting blood samples were taken from each subject on days 0, 28 and 56. An honorarium of 15 dollars was paid to each subject at completion of the study.

II Experimental Protocol

The study protocol was approved by the Faculty of Human Ecology Ethics Committee and the Health Sciences Center Medical Ethics Committee and written consent was obtained from each subject. A copy of the consent form used in the study is shown in Appendix 1. Medications were reviewed with potential subjects. Those on cholesterol lowering drugs were not accepted for participation in the study. However,

subjects on oral hypoglycemic agents (OHA) [10 of 37 subjects] or insulin [2 of 37 subjects] were accepted for participation in the study. Likewise, only individuals who had controlled high blood pressure (BP<150/90 mm Hg) were admitted to the study. Participants were instructed to notify the study coordinator or dietitian of any changes in medications and were instructed to maintain their usual routines and activity levels throughout the study. A total sample size of forty subjects, twenty males and twenty females, was planned for the study. Due to sample capacity limitations of the ultracentrifuge and Milton Roy Spectrophotometer, five groups of eight subjects (one male and female on each of CAN, LLNA, SNFLR, and CONT) were to be run consecutively. Extreme difficulty in subject recruitment resulted in eight groups of between one and eight subjects per group for a total sample size of 37 being recruited for the study. One subject on the LLNA diet dropped out of the study at Week 4 but initial and mid-point data was used in the data analysis. Subjects were randomly assigned to one of the four dietary groups based on gender and randomly stratified according to five age categories (40-45, 46-50, 51-55, 56-60, 61-70 years of age) to distribute age evenly across the four treatments. Study design and subject grouping is outlined in Appendix 2.

III Subjects

Subject recruitment for the study was done initially through the Diabetes Education Centre (DEC) using pamphlets and posters. However, it was necessary to expand the search using the Canadian Diabetes Association information banner and the Manitoba Television Network "What Matters" community advertisement segment.

Potential subjects were interviewed and screened by telephone and relevant information obtained. Subjects chosen to participate in the study were selected based on: the presence of physician diagnosed type II diabetes mellitus; the absence of cholesterol lowering medication; age between 40-70 years; controlled hypertension (blood pressure not exceeding 150/90 mm Hg); BMI between 25-50; reasonable glycemic control; frequency and amount of peanut butter consumed; and perceived willingness to comply with the study protocol. Consent by the subjects physician was obtained before being admitted to the study.

IV Diets

Subjects other than control were provided with one of the three dietary fat sources in the form of a spread (consisting of a blend of 40% butter and 60% oil) and salad oil. Fatty acid composition of the experimental spreads and oils are summarized in Appendix 3 and 4, respectively. Subjects were counseled to maintain a low-fat (30% total energy) diet, as currently recommended by the Canadian Diabetes Association and Health Canada. Evaluation of participant's 3-day food records, collected between weeks 4 and 5 of the study, was done using the Elizabeth Warwick (Elizabeth Warwick B.H.Sc. P.Dt.; Cornwall, PEI; C0A 1H0; 1982-1994) nutrient analysis computer program.

Consumption of experimental spread and oil was monitored using a chart on which subjects recorded their daily intakes. A copy of the chart used to monitor fat intakes is provided in Appendix 5. Mean intakes for each dietary group as recorded by the subjects are summarized in Table 1. Subjects were counseled to use between one and

three teaspoons of spread/oil daily, based on their usual consumption, and to mark any other added fat, such as peanut butter, in a different color marker on the chart.

(a) Handling, Preparation and Storage of Spread and Salad Oil

Spread was blended in a ratio of 40% butter to 60% oil (w/w) just prior to the beginning of each study group and thereafter when needed. It was stored at -10°C until required. The preparation, storage and handling procedures were similar to those utilized in previous studies (Corner, 1989; Chan, 1990; Chard, 1991).

(b) Diet Analysis

The fatty acid content of each experimental spread and oil was determined by gas-liquid chromatography (GLC) following methylation of the fatty acids using sodium methoxide in methanol (Bannon et al, 1985). The fatty acid methyl esters were analyzed with a Hewlett Packard 5890 Gas Chromatograph equipped with a Durabond-225 capillary column, 30m x 0.25 mm, and a film thickness of 0.25 microns (J & W Scientific Inc., Folsom, CA.). Injector, detector, and column temperatures were 250°C, 250°C, and 205°C, respectively. The carrier gas was helium. Peak areas were measured with a Hewlett Packard 3392A integrator. The settings for the gas chromatograph are presented in Appendix 6. Fatty acid peaks were identified using reference samples (cat.#GLC-68B, and GLC-87, Nu-Chek Prep. Inc., Elysian, MN).

V Plasma Lipid and Lipoprotein Cholesterol Analysis

A sample of venous blood was taken from the antecubital vein of each subject following a 12-hour overnight fast on day 0, 28, and 56 of the study. Blood was collected in 10 mL vacutainer tubes containing 0.10 mL of 15% EDTA solution as anticoagulant and immediately chilled on ice. Plasma total cholesterol, HDL-C, LDL-C, triglycerol, and glycosylated hemoglobin (GlyHb) were determined by the Clinical Biochemistry Department of the Health Sciences Centre (HSC) according to the methods described in the HSC Reference Laboratory Manual - Appendix 7 and according to their interpretive guidelines for lipid profiles. A copy of the blood requisition form is shown in Appendix 7. Blood samples transported to the University of Manitoba Fort Garry Campus for LDL separation and analysis were centrifuged using the Beckman TJ centrifuge, with aerosol containment, at 2000 RPM (850 x g) for 15 minutes at 4°C to remove the red blood cells. The plasma was then transferred to an acid washed, 5 mL screw-top vial and stored at 4°C for later lipoprotein separations. Lipoprotein separations were initiated on the first day of blood sample collection and was completed over a three day period.

(a) Lipoprotein Separation

Plasma lipoprotein fractions were separated by continuous density ultrcentrifugation (Lindgren, 1975). Initially, 1.5 mL of plasma was centrifuged, using a 50 Ti rotor, in a salt (NaCl) solution (density 1.0630 gm/mL) for 18 hours at 104,000 x g (34,000 RPM)

and 18°C to separate the VLDL+LDL fraction from the HDL fraction. The VLDL+LDL layer was removed and brought to 1.0 mL in volumetric tubes using the 1.0630 gm/mL salt (NaCl) solution. To isolate the LDL fraction, 0.9 ml of the VLDL+LDL was centrifuged in a medium of density 1.0063 g/mL for 18 hours at 104,000 x g and 18°C (Kita et al, 1987). Duplicate samples of LDL were prepared for all blood samples; one for LDL fatty acid composition analysis and the other for LDL conjugated diene production and LDL particle size determination.

(b) LDL Dialysis

Spectra/Por 4 Molecular Porous Dialysis Membrane tubing (10 mm wide; 12,000 to 14,000 M.W; Spectrum, Houston Texas) was cut into 12.5 cm lengths (one length per sample) and soaked in distilled water for 30 minutes prior to filling with the LDL fraction. The LDL fraction was transferred to the dialysis tubing and both ends sealed with clamps. Dialysis tubes were placed into capped amber bottles to which cold phosphate buffer saline (PBS) was added at a ratio of approximately 250 ml PBS per dialysis tube, with a maximum of four tubes per bottle. Oxygen was removed from the PBS by purging with N_2 gas for about 5 minutes. Dialysis bottles were placed on a magnetic stirrer in the cold room (temperature 4°C) and dialyzed in the dark for 22 hours to remove EDTA, the anticoagulant (chelating agent) present in the vacutainer tubes used

for blood collection. The PBS was changed twice during dialysis at three and six hours utilizing the same oxygen purging procedure each time (Jialal and Grundy, 1992).

(c) LDL Conjugated Diene Analysis

The protein concentration of the dialyzed LDL fraction was determined by the method of Lowry et al (1951) and adjusted to 250 micrograms (μg) LDL protein per mL. Oxidation was initiated by combining 0.1 mL dialyzed LDL with 0.9 mL PBS containing 5.55 micromoles copper (final solution concentration was 5 micromoles Cu per 1.0 mL) in a glass cuvette. LDL oxidation rate was determined by monitoring the change in absorbance at 234 nm and 37°C on a Milton Roy UV Spectrophotometer equipped with an eight-position automatic sample changer, allowing for the determination of up to seven samples at the same time (one cuvette served as a blank each time). Absorbance was recorded every five minutes for 3 to 4 hours depending upon when each sample reached the plateau phase of diene production (Jialial and Grundy, 1992). The indices of maximal rate of oxidation (diene production rate) and the total amount of dienes produced (initial absorbance subtracted from the highest absorbance recorded; i.e., the point at which absorbance plateaued) was determined from the kinetic absorbance profile of each individual LDL preparation. These indices were assumed to describe the oxidative susceptibiliy of the LDL fraction for each subject.

(d) LDL Phospholipid Analysis

A sample of dialyzed LDL (250 μg LDL protein per mL) was extracted by the method of Folch et al (1957) to separate the phospholipids from the inorganic phosphorous. In brief, the procedure involved the addition of 3 mL 2:1 chloroform:methanol to 0.3 mL dialyzed LDL to which, 0.7 mL of 0.88% potassium chloride was added. The solution was centrifuged at 2000 RPM in a Beckman GS-6 centrifuge for ten minutes and the top layer removed and disgarded. One mL of the chloroform layer was removed and placed into a 12 x 75 mm disposable glass vial which was capped and stored in a freezer at -20°C for later phospholipid analysis. Phospholipids were determined by the method of Bartlett (1959). In brief, the tubes were removed from the freezer, placed in a 37°C water bath and evaporated to dryness under a stream of nitrogen. One mL of water and 0.25 mL of 10 N H_2SO_4 were added to the tubes and the tubes were placed in a 150-160°C oven for three hours. Two drops of hydrogen peroxide was added to each tube and the tubes were heated in the oven for another 1.5 hours. The tubes were removed from the oven and allowed to cool. The color reaction phase of the analysis was initiated by adding 2.15 mL water to each sample. Blanks and standards were prepared as follows: (1) blank consisted of 2 mL water and 0.25 mL 10N H_2SO_4; and (2) standard consisted of 1 mL of a 2 μg/mL stock standard (KH_2PO_4) plus 1 mL of water and 0.25 mL 10 N H_2SO_4. Fiske reagent and 5% ammonium molybdate (0.1 mL of each) were added to each tube (blank, standards, and samples). The tubes were then placed in a boiling water bath for 7 minutes, and after this

period of time, removed and allowed to cool to room temperature. Absorption was read on the Milton Roy UV Spectrophotometer at 830 nm using a water blank to zero the instrument. Blank readings were subtracted from all other readings, and the amount of phospholipid present in the LDL determined (see Appendix 8 for calculations).

(e) LDL Particle Size Determination

Protein concentration of the LDL fraction was determined by the method of Lowry et al (1951) and adjusted to 250 micrograms per mL of LDL. Using this LDL solution, total and free (unesterified) LDL cholesterol were determined enzymatically by a modification of the method of Allain et al (1974) using Sigma Diagnostics Cholesterol Reagent kits. LDL triglycerides were analyzed using a colorimetric, GPO (L-α-glycerol phosphate oxidase) Triglyceride Assay Kit (Diagnostic Chemicals Limited, Charlottetown, PEI) according to the enzymatic method of Fossatti and Lorenzo (1982) as modified by McGowan et al (1983). The phospholipid content of the LDL fraction, along with the values for LDL protein and triglyceride content and LDL total and free cholesterol content were used to calculate LDL particle size by the equations developed by Van Heek and Zilversmit (1991; see equations in Appendix 9).

(f) LDL Fatty Acid Analysis

LDL samples were saponified by incubation of 0.3 mL sample with 1.8 mL 0.4 M KOH in 92% ethanol (v/v) at 37°C for 1.5 hours. After incubation, 2.8 mL water, 0.8 mL ethanol, and 200 µL internal standard (pentadecanoic acid; C15:0) were added to each tube. Sterols were extracted twice with 5 mL *n*-hexane and disgarded. The saponified samples were acidified with 100 µL 6N HCl and extracted twice with 6 mL *n*-hexane. Six mL of the hexane extracted from each sample was added to screw top glass tubes, evaporated under N_2 (g) in a 37°C water bath, combined with 3 mL methanolic HCl, and incubated at 80° C for 30 minutes. Samples were cooled to room temperature, combined with 500 µL of petroleum ether and 1 mL water, and centrifuged for two to three minutes. The petroleum ether layer was removed into a GLC vial and evaporated under N_2 gas. The fatty acid methyl esters were dissolved in 100 µL petroleum ether, flushed with N_2 gas and capped. One microlitre aliquots of each sample were analyzed for fatty acids by gas chromatography on a 30 m x 0.25 mm i.d. Durabond-225 capillary column (J & W Scientific Inc., Folsom, CA) with a film thickness of 0.25 microns. Analysis was performed on a 5890 GC (programmed temperature vaporizer system) equipped with a flame ionization detector (FID) and a Hewlett Packard 3392A integrator. The carrier gas used was helium. The sample volume injected on column was 1 µL (splitless injection). Temperature of the column was programmed from 70 °C to 180 °C at a rate of 20 °C/min. and then from 180 °C to 220 °C at a rate of 3 °C/min. Injector and detector

temperatures were 200 °C and 260 °C, respectively. Quantitation of each fatty acid was based on peak area compared with the peak area for the C15:0 internal standard.

VI Statistical Analysis

Statistical analysis of the data was performed using SAS statistical software (SAS Institute Inc., Cary NC 1984,1986). Analysis of Variance (ANOVA) was performed to determine significant differences among the dietary treatments CAN, LLNA, SNFLR, and CONT and to identify diet x gender interactions for the study periods 0-56, 0-28, and 28-56 days, respectively. When interactions were found, linear contrasts were performed to determine significance. Diet effects were determined using both Duncan's (DMCT) and Tukey's (TMCT) multiple comparison tests.

RESULTS

I Subjects

Thirty-six type II diabetic subjects completed the 56-day study. Subject compliance and motivation was monitored by 3-day food records, a take home fill-in fat intake chart, weight records, and personal contact with the study's coordinator and a Registered Dietitian. Of the total 37 subjects in the study, 13 (5 males and 8 females) controlled blood glucose using an oral hypoglycemic agent (OHA; glyburide), 2 used insulin (one male and one female), and 22 (12 males and 10 females) controlled blood glucose by diet modification alone. In general, the subjects maintained constant body weight throughout the study. One male and one female lost weight (2.7 and 5.9 kg) while two female subjects gained weight (1.5 and 2.0 kg). These weight changes occurred at a slow rate over the duration of the study. Weight loss was attributed to improved meal eating patterns and food choices and to a more concerted effort to follow dietary recommendations, and not due to caloric restriction. Weight gain, for one subject, was attributed to weight training classes, while weight gain for the other is unexplained. Table 1 shows the mean age, BMI, and fat intakes for the experimental groups. Subject age did not differ significantly among the dietary groups; the mean age of the CAN, LLNA, SNFLR, and CONT groups were 54.0 ± 8.2, 52.5 ± 6.9, 54.8 ±10.3, and 52.6 ± 7.2 years, respectively. The mean BMI and standard deviation for each of the dietary groups was 31.7 ± 6.6, 31.0 ± 9.8, 31.4 ± 8.6, and 31.3 ± 5.7 for CAN, LLNA, SNFLR,

and CONT, respectively. No significant differences in BMI ($p>0.05$) were found among the dietary groups.

(a) Subject 3-day Food Record and Fill-In Fat Chart Analysis

The 3-day food records of each subject were analyzed for dietary parameters including the percentage of daily fat intake from the experimental spread and oil (Table 1). The average daily energy was determined along with the percent of total energy from protein (PRO), carbohydrate (CHO), and total, saturated, monounsaturated, and polyunsaturated fat. The average daily cholesterol intake was also determined (Table 1). No significant differences among the dietary groups were found for average energy intake or the percent of daily energy from PRO, CHO, total fat, saturated fat and monounsaturated fat. Polyunsaturated fat intake was significantly higher ($p<0.05$) on the SNFLR than on the CONT diet; the average percentage of daily total energy ingested in the form of PUFA was 6.8 ± 2.5 % and 4.4 ± 0.7 % for the SNFLR and CONT diets, respectively. Significant gender differences were found for the percent of daily energy from PRO; men derived a higher proportion of energy from PRO than women (21.4 ± 2.8 % vs 19.2 ± 3.1 % for men and woman, respectively; $p<0.04$). However, women derived a higher proportion of their daily energy intake from CHO (44.4 ± 7.6 % vs 49.4 ± 4.4 % for men and women, respectively; $p<0.02$). Analysis of the 3-day food records showed the average fat intake for all subjects was 33.8 ± 2.4 % of daily energy as fat. The average total fat intake in the form of experimental spreads and oils was 13.4% of total fat intake which did not differ significantly among the experimental dietary groups.

Table 1. Mean Subject and Dietary Data for the Experimental Groups

	Experimental Diet Group [1]					
Variable [2]	CAN	LLNA	SNFLR	CONT	Male	Female
Age	54.0 ± 8.2	53.7 ± 6.5	54.8 ± 10.3	53.3 ± 7.4	-	-
BMI (Kg/m^2)	31.7 ± 6.6	28.7 ± 8.03	31.4 ± 8.6	31.6 ± 6.0	-	-
Spread/Oil Intake (g/wk)	66.5 ± 24.5	68.0 ± 29.5	59.5 ± 19.75	75.0 ± 15.5	-	-
Kcal/day	1770 ± 415	1614 ± 432	1687 ± 457	1439 ± 369	-	-
% PRO [3]	20.01 ± 3.33	18.12 ± 4.04	20.86 ± 1.41	21.24 ± 3.13	21.39 ± 2.80	19.15 ± 3.11
%Total Fat	32.84 ± 7.57	37.37 ± 4.09	32.90 ± 9.41	32.14 ± 5.24	-	-
% CHO [4]	47.47 ± 7.07	47.09 ± 1.36	46.84 ± 9.27	47.25 ± 5.53	44.42 ± 7.62	49.35 ± 4.40
% SAT	10.73± 3.05	11.85 ± 2.60	10.16 ± 3.02	11.48 ± 2.04	-	-
% MONO	12.99 ± 3.44	15.55 ± 2.83	12.26 ± 4.63	12.77 ± 2.51	-	-
% POLY [5]	5.30 ±1.62 ^{xy}	6.60 ± 1.42 ^{xy}	6.78 ± 2.47 ^{x}	4.39 ±0.72 ^{y}	-	-
CHOL	309 ± 179	268 ± 90	230 ± 100	237 ± 68	-	-

[1] Experimental dietary groups are as follows: CAN- regular canola oil; LLNA- low linolenic acid canola oil; SNFLR- sunflower oil; CONT- control.

[2] All values are mean ± SD.

[3] Significant gender differences ($p<0.04$).

[4] Significant gender differences ($p<0.02$).

[5] Significant diet differences ($p<0.05$). Values in the same row with the same superscript letter do not differ.

Self-recorded fat intakes were used to determine the total amount of experimental spread and oil ingested over the study period as well as the average weekly intakes of spread and oil for each subject. Mean spread and oil intake per week for each dietary group was 66.5 ± 24.5, 68.0 ± 29.5, 59.5 ± 19.75, and 75.0 ± 15.5 grams for CAN, LLNA, SNFLR, and CONT, respectively and did not differ significantly ($p>0.05$) among the groups. The average percent of oleic acid intake, determined from the 3-day food records, was 13.0 ± 3.4%, 15.6 ± 2.8%, 12.3 ± 4.6%, and 12.8 ± 2.5% for the experimental dietary groups CAN, LLNA, SNFLR, and CONT, respectively, and did not differ significantly. The percent linolenic acid present in the experimental spreads was 7.7%, 2.2%, and 0.4% for the CAN, LLNA, and SNFLR diets, respectively (Appendix 3). The three experimental spreads provided approximately 13.1 mg cholesterol/tsp spread. Mean daily intake of cholesterol varied from 230 to 309 mg but did not differ significantly among the dietary groups or between men and women (Table 1).

(b) Subject Glucose Control (Glycosylated Hemoglobin)

Mean GlyHb levels (expressed as a percent of total hemoglobin, Hb_{A1C}) for the dietary groups at Day 0 were 9.9 ± 3.8%, 8.8 ± 2.0%, 9.2 ± 1.4%, and 8.9 ± 2.2% for the CAN, LLNA, SNFLR, and CONT diets, respectively. At Day 0, seven subjects were within the normal range of GlyHb (4.3-7.0%), whereas 30 subjects were above the threshold level; the number of subjects with GlyHb levels from 7-10% and above 10% were 19 and 11, respectively. There were no differences in mean change in GlyHb levels due to the experimental diets for the study periods 0-56, 0-28, and 28-56 days (Table 2).

Table 2. Mean Change in Glycosylated Hemoglobin Levels on Experimental Diets [1,2]

	Study Period (Days) [3]		
Variable	**0-56**	**0-28**	**28-56**
CAN	-0.57 ± 1.83 [a]	-0.13 ± 0.78 [a]	-0.44 ± 1.32 [a]
LLNA	-0.14 ± 0.89 [a]	-0.34 ± 0.69 [a]	0.21 ± 0.94 [a]
SNFLR	0.02 ± 1.36 [a]	-0.19 ± 0.88 [a]	0.02 ± 0.93 [a]
CONT	-0.04 ± 1.81 [a]	0.16 ± 1.67 [a]	-0.20 ± 0.50 [a]
Males [4]	-0.81 ±1.43	-	-
Females	0.30 ± 1.40	-	-

[1] All values are mean ± SD. F-values and associated probabilities for the statistical comparison among experimental diets are shown in Appendix 10.

[2] Means in the same column with the same superscript letter do not differ ($p>0.05$).

[3] Baseline (Day 0) values for GlyHb level (%) were 9.9, 8.8, 9.2, and 8.9 for the CAN, LLNA, SNFLR, and CONT groups, respectively.

[4] Significant ($p<0.03$) gender differences during the study period 0-56 days.

Significant differences ($p<0.03$) in the change in mean GlyHb level were found between men and women over the study period 0-56 days. During this period, mean GlyHb levels for men decreased 8.4%, while the mean level for women increased 3.4% (Table 3).

II Effect of the Experimental Diets on Plasma Total and Lipoprotein Cholesterol Concentrations of NIDDM Subjects

Mean concentrations of plasma total cholesterol (TC) and lipoprotein (LP) cholesterol at Day 0 of the study and the percent changes in these levels in response to the experimental diets for the study periods 0-56 days are summarized in Tables 4 and 5, respectively. The mean change in plasma TC and LP cholesterol concentration (mmol/L) for the three study periods: 0-56, 0-28, and 28-56 days are summarized in Tables 6, 7, and 8, respectively.

Plasma TC concentrations were not affected by the experimental diets over the duration of the study (0-56 days; Tables 5 and 6; Figure 3) or over the intervals 0-28 and 28-56 days (Tables 5 and 6). Similarly, mean change in TC and LDL-C concentrations were unaffected by the experimental diets for the study periods 0-28 and 28-56 days (Tables 7 and 8). However, over the study period 0-56 days, a significant ($p<0.05$) diet x gender interaction was found for LDL-C. The mean LDL-C concentration for women did not change on the CAN diet and differed significantly ($p<0.03$) from that on the CONT diet which decreased 23.5% (Figure 4; Tables 5 and 6). By contrast, mean LDL-C concentrations decreased 7.3% on the CAN diet and increased 8.8% on the CONT diet for men ($p<0.04$). For women, the change in mean LDL-C concentration on the LLNA or

Table 3. Mean Glycosylated Hemoglobin Levels (Day 0) and Percent Change (0-56 Days) for Males and Females

	GlyHb (%)	
Gender	Mean [1,2]	Percent Change [3] (0-56 days)
Male	9.62 ± 3.21	- 8.41
Female	8.85 ± 1.63	3.38

[1] All values for mean glycosylated hemoglobin levels are mean ± SD at Day 0.

[2] There were significant gender differences ($p<0.05$) in mean glycosylated hemoglobin levels during the 0-56 day study period.

[3] Values expressed as a percent increase or decrease for the study period 0-56 days and are based on the mean values shown at Day 0.

Table 4. Mean TC and LP Concentrations for the Experimental Diet Groups (Day 0) [1,2]

		Diet Group			
		CAN	LLNA	SNFLR	CONT
Plasma Lipid Parameter [3]	Normal Range (mmol/L)[4]				
TC	2.95-5.20	5.46 ± 0.96 [a]	5.64 ± 0.57 [a]	5.56 ± 1.06 [a]	5.44 ± 1.09 [a]
LDL-C [5]	1.68-3.40				
M		3.17 ± 0.57	3.26 ± 0.76	3.57 ± 1.16	2.87 ± 0.32
F		3.11 ± 1.08	3.17 ± 0.57	3.72 ± 0.86	3.65 ± 1.18
HDL-C	0.9-2.0	1.28 ± 0.27 [a]	1.34 ± 0.39 [a]	1.32 ± 0.31 [a]	1.34 ± 0.23 [a]
TAG	0.3-2.3	2.26 ± 1.21 [a]	2.37 ± 0.76 [a]	1.42 ± 0.52 [a]	2.42 ± 1.34 [a]
TC:HDL-C	0-4.5	4.50 ± 1.45 [a]	4.42 ± 0.90 [a]	4.41 ± 1.14 [a]	4.88 ± 1.04 [a]

[1] All values are mean ± SD.

[2] Means in the same row with the same superscript letter do not differ ($p>0.05$).

[3] Units for plasma lipid parameters are mmol/L except for the TC:HDL-C ratio.

[4] Health Sciences Centre Reference Laboratory Manual- Appendix 7 (Interpretive Guidelines for Lipid Profiles).

[5] Significant ($p<0.05$) diet x gender interaction.

Table 5. Percent Change in TC and LP Concentrations for the Experimental Diet Groups: 0-56 Days [1,2]

	Diet Group			
	CAN	LLNA	SNFLR	CONT
Plasma Lipid Parameter				
TC	-3.05 [a]	-7.36 [a]	-0.24 [a]	-6.78 [a]
LDL-C [3]				
M	-7.25 [b]	-5.20 [b]	-5.39 [b]	8.82 [a]
F	0.21 [ab]	-11.74 [b]	-2.58 [b]	-23.51 [b]
HDL-C	-0.31 [ab]	8.00 [a]	6.40 [a]	7.62 [a]
TAG	15.74 [a]	-20.86 [a]	0.18 [a]	-12.89 [a]
TC:HDL-C	0.58 [a]	-5.52 [a]	-4.59 [a]	-13.15 [a]

[1] All values are percent change from baseline (Day 0).

[2] Means in the same row with the same superscript letter do not differ ($p>0.05$).

[3] Significant ($p<0.05$) diet x gender interaction.

Table 6. Mean Change in Plasma Total and Lipoprotein Cholesterol Concentrations on Experimental Diets: 0-56 Days [1,2]

	Diet Group			
Plasma Lipid (mmol/L)	**CAN**	**LLNA**	**SNFLR**	**CONT**
TC	-0.17 ± 0.97 [a]	-0.42 ± 0.39 [a]	-0.01 ± 0.87 [a]	-0.37 ± 1.21 [a]
LDL-C [3]				
M	-0.23 ± 0.18	-0.17 ± 0.63	-0.19 ± 0.93	0.25 ± 0.52
F	0.01 ± 0.46	-0.37 ± 0.33	-0.10 ± 0.81	-0.86 ± 1.42
HDL-C	-0.004 ± 0.10 [a]	0.11 ± 0.36 [a]	0.08 ± 0.19 [a]	0.09 ± 0.20 [a]
TAG	0.36 ± 0.60 [a]	-0.49 ± 1.06 [a]	0.003 ± 0.32 [a]	-0.31 ± 0.86 [a]
TC:HDL-C	0.03 ± 0.29 [a]	-0.24 ± 0.71 [a]	-0.20 ± 0.79 [a]	-0.64 ± 1.12 [a]

[1] All values are mean ± SD. F-values and associated probabilities for the statistical comparison among experimental diets for all above lipid parameters are shown in Appendix 11.

[2] Means in the same row with the same superscript letter do not differ ($p>0.05$).

[3] Significant ($p<0.05$) diet x gender interaction. Linear contrasts and associated t-statistic and probability values are shown in Appendix 12a.

Table 7. Mean Change in Plasma Total and Lipoprotein Cholesterol Concentrations on Experimental Diets: 0-28 Days [1,2]

	Diet Group			
Plasma Lipid (mmol/L)	**CAN**	**LLNA**	**SNFLR**	**CONT**
TC	0.06 ± 0.64 [a]	0.15 ± 0.59 [a]	0.06 ± 0.79 [a]	-0.01 ± 1.30 [a]
LDL-C	0.01 ± 0.61 [a]	0.10 ± 0.79 [a]	0.03 ± 0.67 [a]	0.06 ± 1.30 [a]
HDL-C [3]				
M	0.02 ± 0.04	-0.04 ± 0.10	0.03 ± 0.07	-0.02 ± 0.09
F	-0.04 ± 0.08	0.20 ± 0.27	-0.03 ± 0.10	0.06 ± 0.13
TAG [4]	-0.01 ± 0.41 [a]	-0.12 ± 1.20 [a]	-0.08 ± 0.38 [a]	-0.17 ± 0.90 [a]
TC:HDL-C [3]				
M	-0.34 ± 0.54	0.94 ± 1.00	-0.18 ± 0.37	0.71 ± 0.92
F	0.43 ± 0.31	-0.49 ± 0.53	0.16 ± 0.49	-0.56 ± 1.05

[1] All values are mean ± SD. F-values and associated probabilities for the statistical comparison among experimental diets are shown in Appendix 11.

[2] Means in the same row with the same superscript letter do not differ ($p>0.05$).

[3] Significant ($p<0.03$) diet x gender interaction. Linear contrasts and associated t-statistic and probability values are shown in Appendix 12b and 12c.

[4] Significant ($p<0.05$) gender differences. Mean change in TAG levels are 0.20 ± 0.62 and -0.34 ± 0.80 for men and women, respectively. F-values and associated probabilities for the statistical comparison between genders are shown in Appendix 11.

Table 8. Mean Change in Plasma Total and Lipoprotein Cholesterol Concentrations on Experimental Diets: 28-56 Days [1,2]

	Diet Group			
Plasma Lipid (mmol/L)	CAN	LLNA	SNFLR	CONT
TC	-0.25 ± 0.94 [a]	-0.27 ± 0.38 [a]	-0.07 ± 0.72 [a]	-0.36 ± 0.61 [a]
LDL-C	-0.03 ± 0.41 [a]	-0.19 ± 0.24 [a]	-0.17 ± 0.58 [a]	-0.41 ± 0.49 [a]
HDL-C	0.00 ± 0.12 [a]	0.01 ± 0.12 [a]	0.09 ± 0.17 [a]	0.06 ± 0.20 [a]
TAG	0.32 ± 0.53 [a]	-0.19 ± 0.56 [a]	0.09 ± 0.52 [a]	0.15 ± 0.41 [a]
TC:HDL-C [3]	-0.08 ± 0.55 [b]	-0.16 ± 0.52 [b]	-0.21 ± 0.73 [ab]	-0.64 ± 0.71 [a]

[1] All values are mean ± SD. F-values and associated probabilities for the statistical comparison among experimental diets are shown in Appendix 11.

[2] Means in the same row with the same superscript letter do not differ ($p>0.05$).

[3] Significant ($p<0.04$) diet effects. DMCT and TMCT are shown in Appendix 13. Superscript letter assignment is according to TMCT; means in the same row with the same superscript letter are not statistically different ($p>0.05$). F-values and associated probability values for the statistical comparison among experimental diets is shown in Appendix 11.

Figure 3. Effect of Dietary Fat Source on Change in Total Serum Cholesterol: 0-56 Days

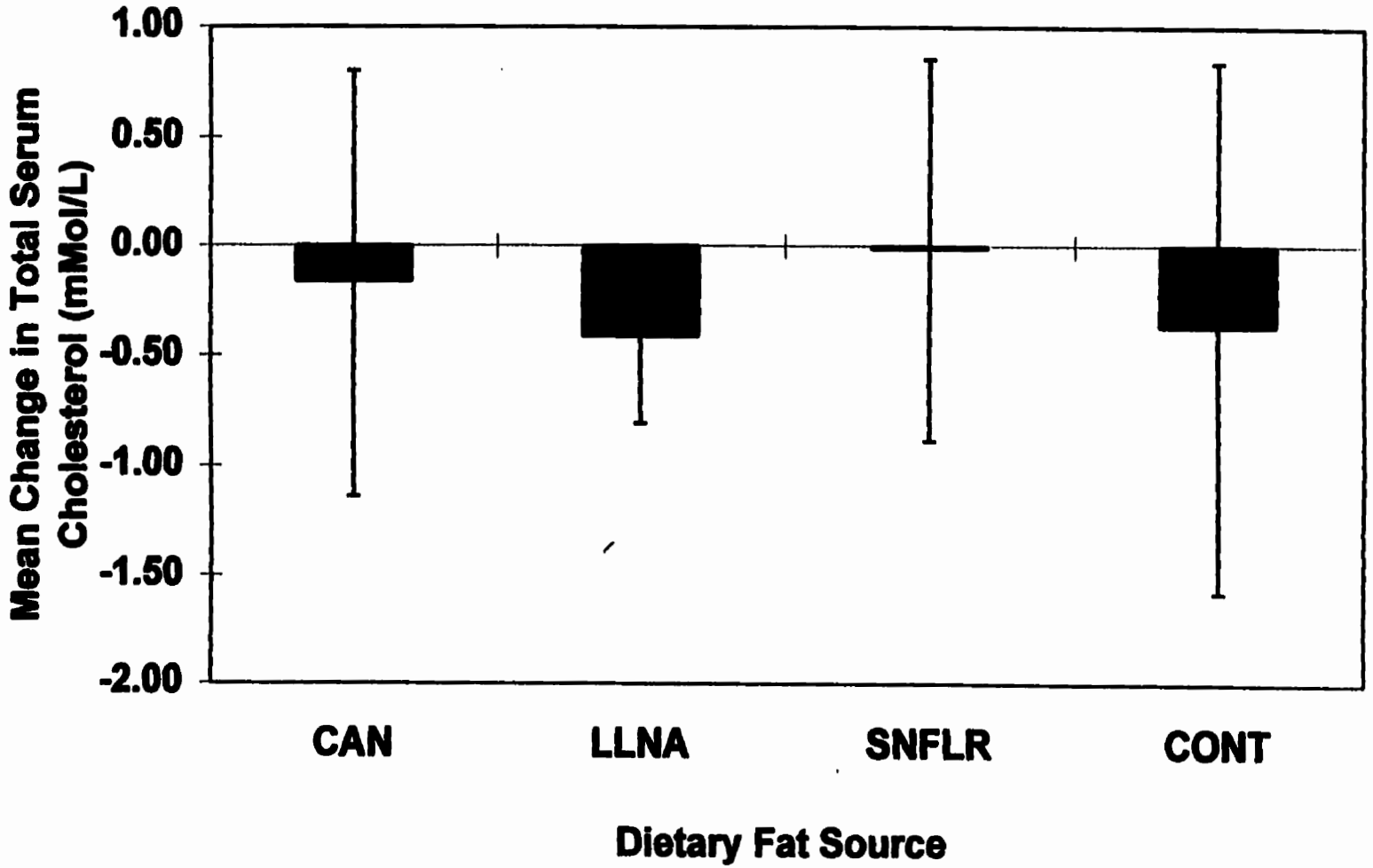
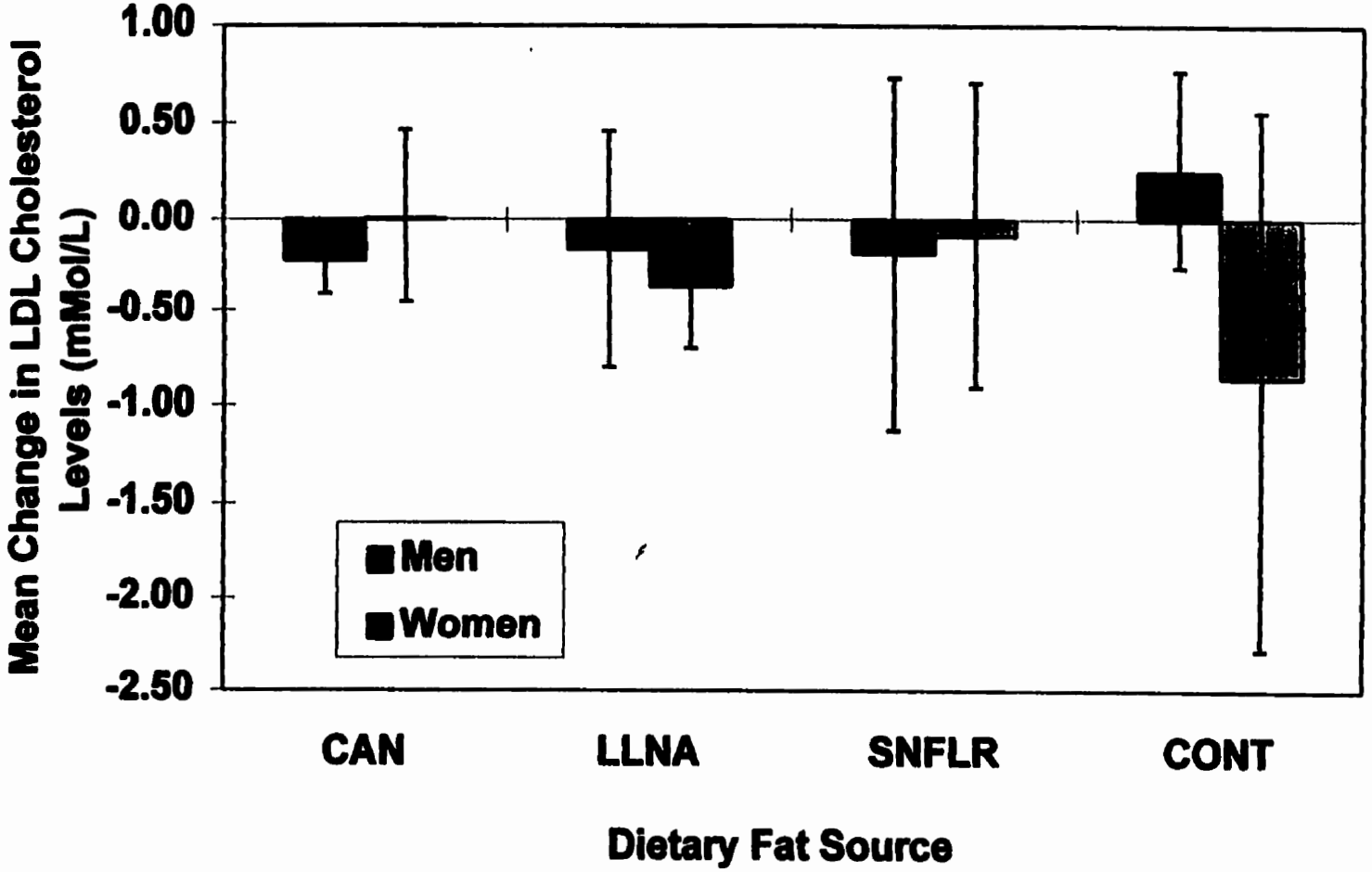


Figure 4. Effect of Dietary Fat Source on Change in LDL Cholesterol in Men and Women: 0-56 Days



SNFLR diets did not differ from the change on the CONT diet (i.e. LLNA ≡ SNFLR ≡ CONT). By contrast, the mean change in LDL-C concentrations on the LLNA and SNFLR diets for men followed the same pattern as for the CAN diet; there was a decrease in LDL-C levels on the CAN (-7.3%), the LLNA (-5.2%;) and SNFLR (-5.3%) diets and an increase on the CONT diet (8.8%). The mean change in LDL-C concentrations in men on the CAN, LLNA, and SNFLR diets differed from the change on the CONT diet (Table 5).

A similar diet x gender interaction ($p<0.03$) was found for HDL-C during the period 0-28 days (linear contrast are presented in Appendix 12b). Table 9 and Figure 5 show that for women, mean HDL-C concentration increased significantly on the LLNA diet (12.7%; $p<0.01$) whereas it decreased on the SNFLR and CAN diets (-2.5 and -2.6 %, respectively). Although the change in mean HDL-C concentration for women also increased on CONT, it did not differ from the changes on the other diets although the p value for the linear contrasts comparing the LLNA and CONT diets was 0.08 (Appendix 12b). No significant differences in the mean change in HDL-C concentrations were found for men among the experimental diets. However, some opposite diet effects for men and women are of interest (Table 9 and linear contrasts in Appendix 12b). Mean HDL-C concentration increased for women on the LLNA diet but decreased on the CAN and SNFLR diets resulting in a significant difference in the effect of the LLNA diet compared to CAN and SNFLR diets. By contrast, the effects of these diets tended to be opposite for men although the changes did not differ significantly among the three diets.

Table 9. Mean HDL-C Concentrations (Day 0) and Percent Change (0-28 Days) for Experimental Diets [1]

	Diet Group			
	CAN	LLNA	SNFLR	CONT
Mean [2] (mmol/L)				
M	1.20 ± 0.19	1.04 ± 0.15	1.48 ± 0.38	1.04 ± 0.24
F	1.36 ± 0.33	1.59 ± 0.34	1.19 ± 0.19	1.22 ± 0.21
% Change [3,4]				
M	2.00 [a]	-3.86 [a]	2.20 [a]	-2.17 [a]
F	-2.65 [a]	12.74 [b]	-2.52 [a]	4.93 [ab]

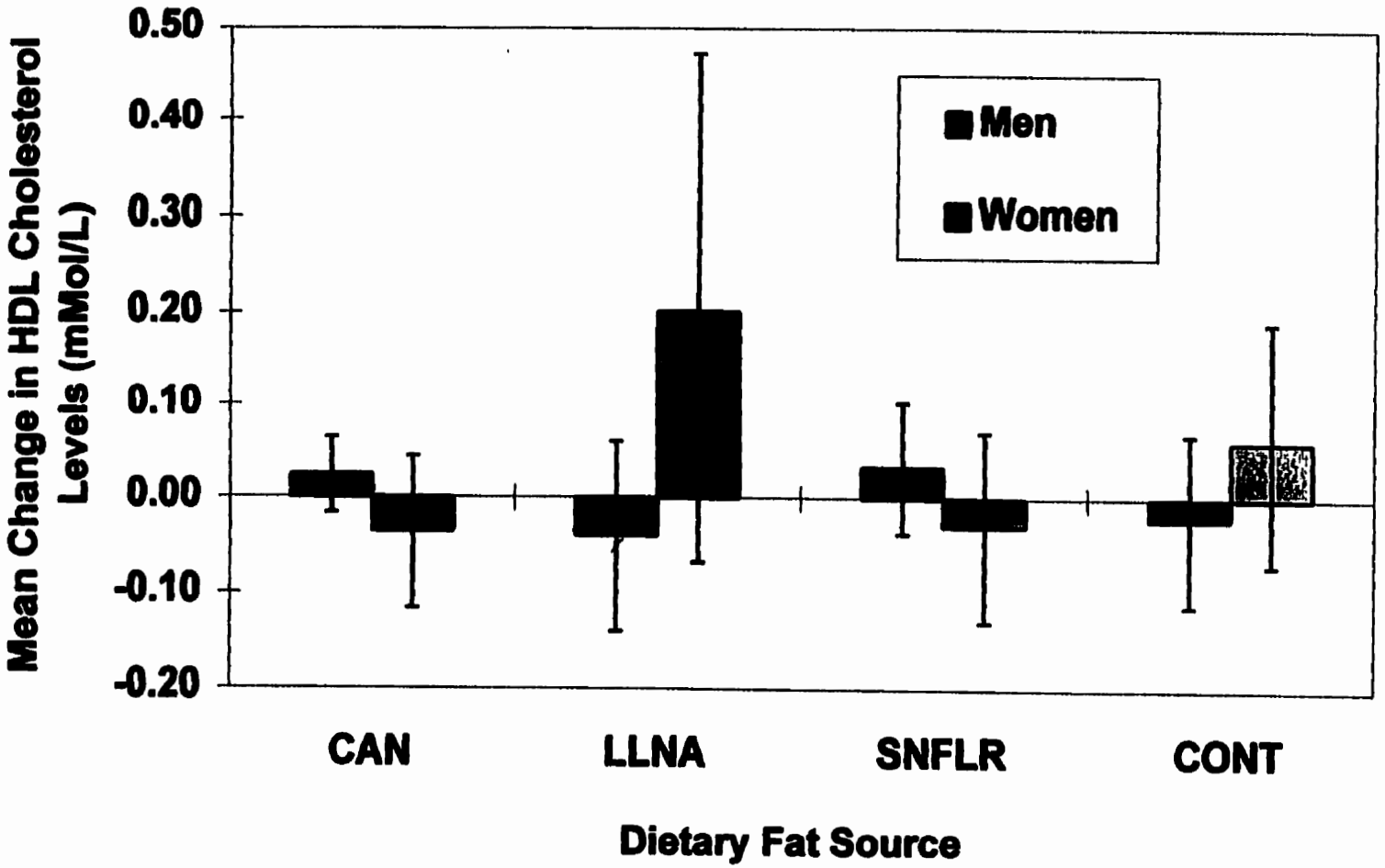
[1] There was a significant ($p<0.03$) diet x gender interaction for HDL-C levels during the 0-28 day study period.

[2] All values for mean HDL-C levels are mean ± SD at Day 0.

[3] Values expressed as a percent increase or decrease for the study period 0-28 days based on the mean values at Day 0.

[4] Values in the same row with the same superscript letter do not differ ($p>0.05$); classification based on linear contrast in Appendix 12b.

Figure 5. Effect of Dietary Fat Source on Change in HDL Cholesterol in Men and Women: 0-28 Days



For the study periods 0-56 and 28-56 days, diet effects on mean change in HDL-C concentrations were negligible.

No dietary effects on TAG levels were found for the three time intervals of the study. However, significant gender differences ($p<0.05$) in mean change in TAG concentrations (Table 7) were found for the period 0-28 days; for women, TAG concentration decreased 14.5% whereas for men TAG concentration increased 10.8% during this period (Table 10 and Figure 6).

Mean change in the ratio of TC:HDL-C was not affected by the experimental diets during the study period 0-56 days, which was consistent with the absence of diet effects on either TC or HDL-C during this period (Table 6). However, there was a significant diet effect ($p<0.04$) on the mean change in the TC:HDL-C ratio during the second interval of the study (28-56 days; Table 8 and Figure 7). The diet effects, in order of largest mean decrease in TC:HDL-C ratio, during days 28-56, were: CONT, -13.2% > SNFLR, -4.8 % > LLNA, -3.5% ≡ CAN, -1.9 % (Table 12). A Duncan's multiple comparison test (DMCT) for diet differences (Appendix 13) showed the CONT diet resulted in a greater decrease in the TC:HDL-C ratio than the CAN, LLNA and SNFLR diets whereas no differences existed among the latter three diets. Tukey's multiple comparison test (TMCT) showed the decrease on the SNFLR diet did not differ significantly from that on the CONT diet (Appendix 13). DMCT and TMCT differ in that Tukey's test is a more conservative test of diet differences and reveals more subtle differences in diet effects.

Table 10. Mean Triglyceride Concentrations (Day 0) and Percent Change (0-28 Days) for Males and Females [1]

Triglycerides		
Gender	**Mean [2] (mmol/L)**	**% Change (0-28 days) [3]**
M	1.85 ± 1.08	10.77
F	2.33 ± 0.98	-14.53

[1] There were significant ($p<0.05$) gender differences in TAG levels during the 0-28 day study period.

[2] All values for mean TAG levels are mean ± SD at Day 0.

[3] Values expressed as a percent increase or decrease for the study period 0-28 days based on the mean values at Day 0.

Table 11. Mean TC:HDL-C Ratio (Day 0) and Percent Change (0-28 Days) for Experimental Diets

	Diet Group			
	CAN	LLNA	SNFLR	CONT
Mean [1,2] (mmol/L)				
M	4.91 ± 1.76	5.09 ± 0.43	3.80 ± 0.96	4.78 ± 1.05
F	4.08 ± 1.08	3.87 ± 0.80	4.89 ± 1.12	4.97 ± 1.15
% Change [3]				
M	-6.82	18.41	-4.73	14.82
F	10.54	-12.65	3.31	-11.22

[1] All values for mean TC:HDL-C levels are mean ± SD at Day 0.

[2] There was a significant ($p<0.05$) diet x gender interaction for the TC:HDL-C ratio during the 0-28 day study period.

[3] Values expressed as a percent increase or decrease for the study period 0-28 days based on mean values at Day 0.

Table 12. Mean TC:HDL-C Ratio (Day 28) and Percent Change (28-56 Days) for Experimental Diets [1]

	D	I	E	T
	CAN	LLNA	SNFLR	CONT
Mean [2] (mmol/L)	4.26 ± 3.88	4.56 ± 1.67	4.42 ± 1.26	4.89 ± 1.24
% Change [3,4]	-1.85 [b]	-3.45 [b]	-4.81 [ab]	-13.23 [a]

[1] There was a significant diet effect ($p<0.04$) for the TC:HDL-C ratio during 28-56 days.

[2] All values for mean TC:HDL-C levels are mean ± SD at Day 28.

[3] Values expressed as a percent increase or decrease for the study period 28-56 days and are based on the above mean values.

[4] Values with the same superscript letter do not differ ($p>0.05$) as classified according to TMCT shown in Appendix 13.

Figure 6. Percent Change in Serum Triglyceride Concentration in Men and Women: 0-28 Days

Figure 7. Effect of Dietary Fat Source on TC:HDL-C Ratio: 28-56 Days

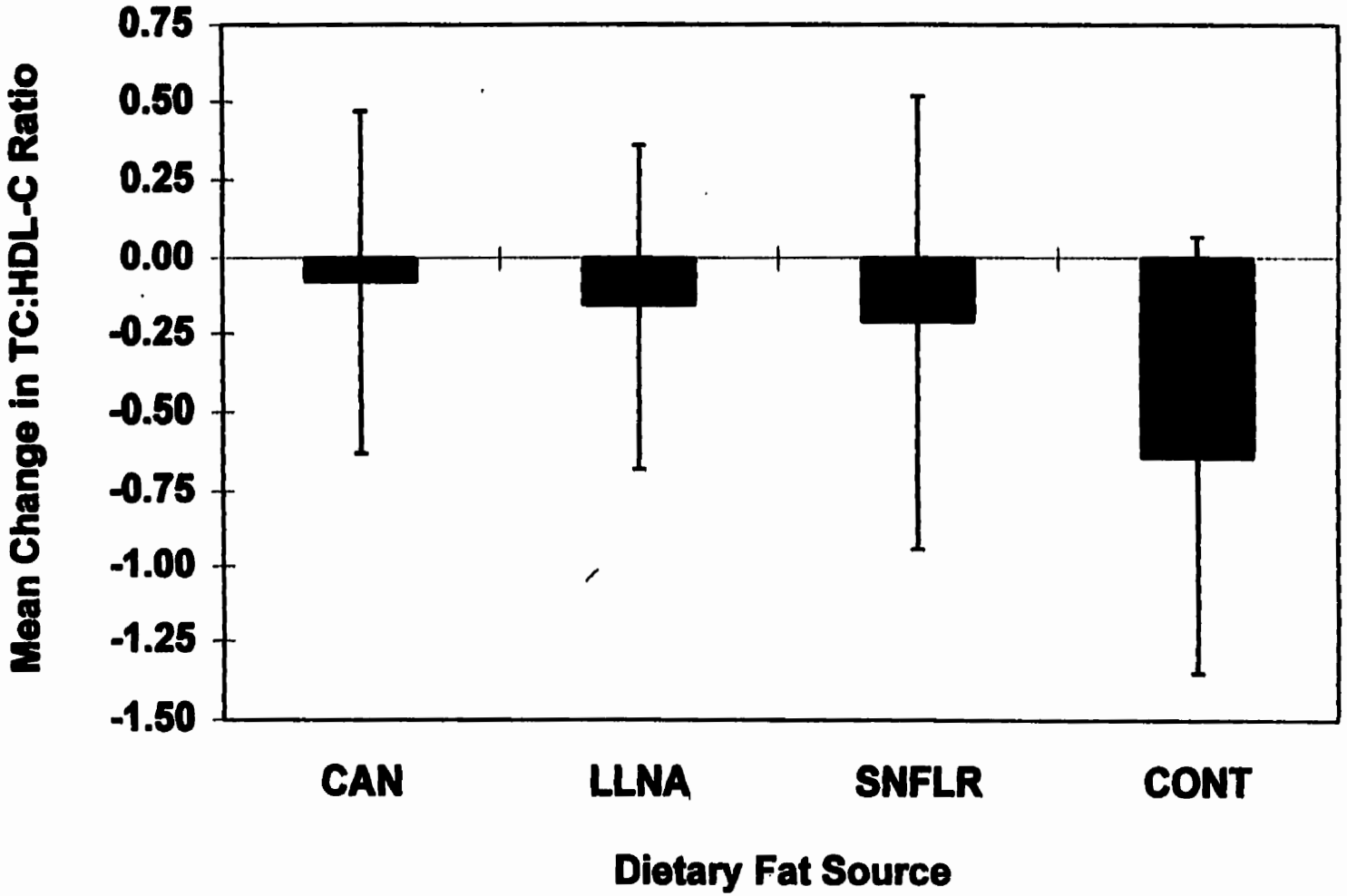
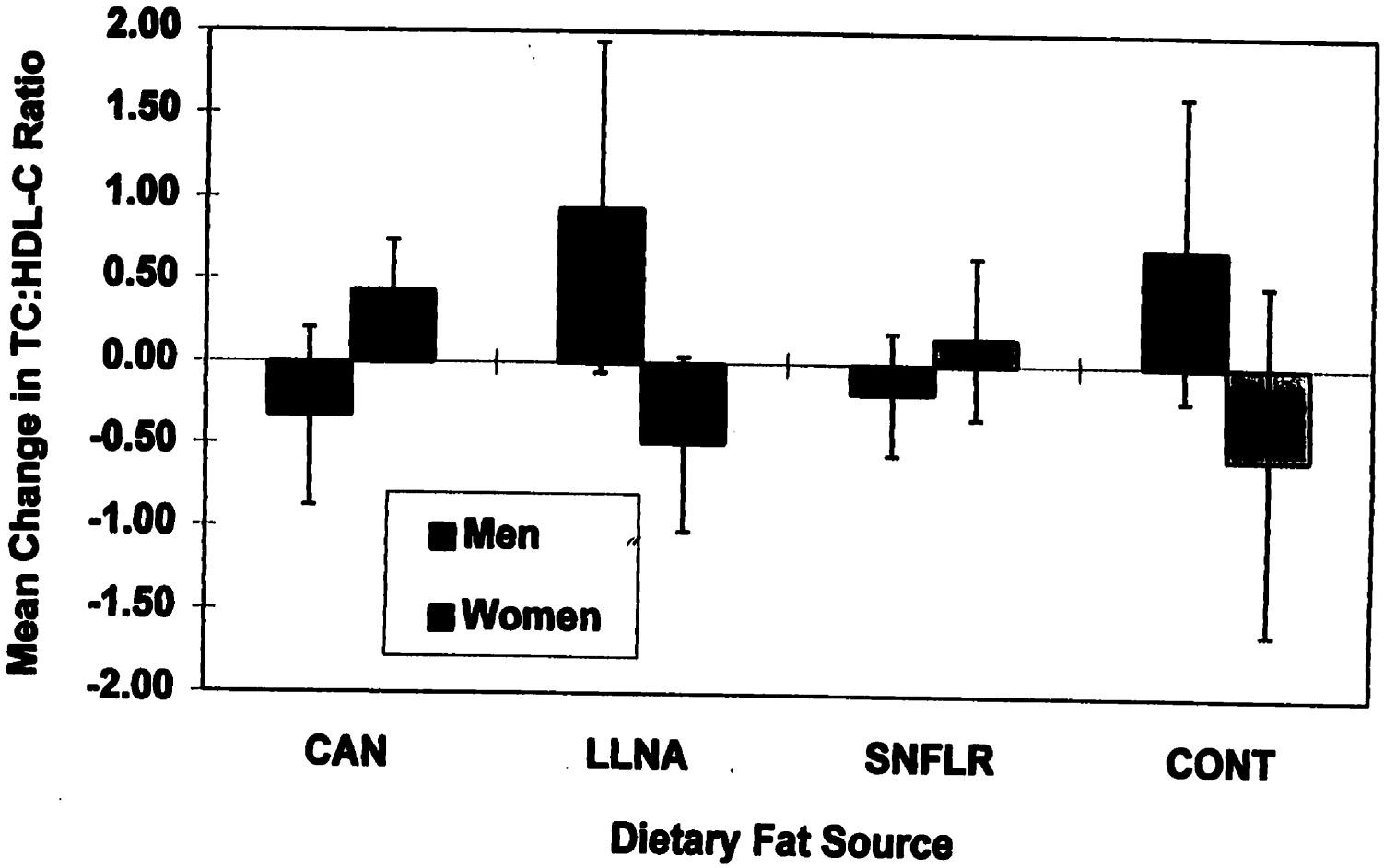


Figure 8. Effect of Dietary Fat Source on TC:HDL-C Ratio in Men and Women: 0-28 Days



Over the first interval of the study (0-28 days), a significant ($p<0.05$) diet x gender interaction occurred in mean TC:HDL-C ratio which coincided with the diet x gender interaction for HDL-C (Table 7). Linear contrasts found no significant differences among the experimental diets for men or for women (Appendix 12c). However, there was a trend towards opposite responses in the TC:HDL-C ratios for men and women on the experimental diets (Table 11 and Figure 8). Compared to the CONT diet where the ratio increased 14.8% for men and decreased 11.2% for women, the TC:HDL-C ratio decreased 6.8% for men and increased 10.5% for women on the CAN diet. A similar pattern prevailed for the comparisons of SNFLR versus CONT; TC:HDL-C ratio on the SNFLR diet decreased 4.7% for men and increased 3.3% for women. By contrast, the change in TC:HDL-C ratio was similar on the LLNA and CONT diets for both men and women, although, in the opposite direction: it increased for men and decreased for women. The TC:HDL-C ratio decreased 12.6% for women and increased 18.4% for men on the LLNA diet. The diet x gender interaction for TC:HDL-C ratio is most likely due to the diet induced changes in HDL-C concentrations for the same study period.

III Effect of the Experimental Diets on LDL Oxidation Rate in NIDDM Subjects

Two indices of LDL oxidation were measured: rate of change (rate) and total change in absorbance (delta absorbance or total conjugated dienes produced) when LDL were oxidized in the presence of a copper catalyst. Data was subjected to the same statistical analysis as the plasma lipid and lipoprotein data. Mean changes in LDL

oxidation rate and the total change in absorbance in response to the experimental diets for all three experimental periods are reported in Table 13a and 13b.

The experimental diets had no affect on LDL oxidation rate for the study period 0-56 days. However, during the first period (0-28 days) of the study, a significant ($p<0.003$) diet x gender interaction occurred. Table 14 summarizes the mean LDL oxidation rate at Day 0 of the study and percent change for the experimental groups. Figure 9 shows the effects of the dietary fat source on the LDL oxidation rate for men and women during the 0-28 day study period. The change in mean LDL oxidation rate for women was greatest for the CONT group (14.2%). The change in rate on the CAN (5.4%) and LLNA (8.7%) diets did not differ from that on the CONT diet for this parameter (i.e. CAN ≡ LLNA ≡ CONT), however, the SNFLR diet resulted in a significantly lower (-2.7%; $p<0.007$) mean LDL oxidation rate than the CAN, LLNA, or CONT diets. The CONT diet also resulted in an increase in the mean change in LDL oxidation rate for men (23.6%). However, unlike the effect for women, there were no changes in mean LDL oxidation rate on the CAN, LLNA and SNFLR diets over the same period. As a result, the change on the CONT diet differed significantly from that on the CAN, LLNA, and SNFLR diets ($p<0.0004$, $p<0.002$ and $p<0.001$, respectively).

Significant ($p<0.04$) differences in LDL oxidation rate, also were found during the second month of the study (28-56 days). Table 15 summarizes the mean LDL oxidation rate at Day 28 of the study and the percent change for this period. Figure 10 shows the effect of dietary fat source on the LDL oxidation rate during this period. A Duncan's multiple comparison test (DMCT) showed that an increase in LDL oxidation rate

Table 13a. Mean Change in LDL Oxidation Rate on Experimental Diets [1,2]

	Diet Group			
	CAN	LLNA	SNFLR	CONT
Rate (ΔAbs/min)				
0-56	-2 ± 23 [a]	-1 ± 7 [a]	10 ± 20 [a]	5 ± 12 [a]
0-28 [3]				
M	-6 ± 18 [a]	0 ± 8 [a]	-2 ± 21 [a]	19 ± 16 [b]
F	6 ± 12 [a]	8 ± 10 [a]	-3 ± 35 [b]	12 ± 6 [a]
28-56 [4,5]	-2 ± 15 [ab]	-6 ± 10 [b]	10 ± 40 [a]	-7 ± 5 [b]

[1] Values for mean change in LDL oxidation rate are the mean and the SD x 10^{-4}.

[2] Means in the same row with the same superscript letter do not differ ($p>0.05$).

[3] Significant ($p<0.003$) diet x gender interaction. Linear contrasts and associated t-statistic and probabilities are shown in Appendix 12g.

[4] Significant diet effects; $p<0.04$ for oxidation rate (28-56 days). DMCT and TMCT for LDL oxidation rate are shown in Appendix 15. F-values for the statistical comparisons among diets are shown in Appendix 14.

[5] Values with the same superscript letter do not differ ($p>0.05$); classification based on DMCT in Appendix 15.

Table 13b. Change in Mean LDL Absorbance (Delta Abs) on Experimental Diets [1,2]

		Diet	Group	
	CAN	LLNA	SNFLR	CONT
Delta Abs				
0-56	10 ± 40^{a}	3 ± 29^{a}	20 ± 30^{a}	20 ± 30^{a}
0-28 [3,4]	2 ± 23^{b}	-2 ± 28^{b}	20 ± 30^{ab}	30 ± 30^{a}
28-56	10 ± 30^{a}	5 ± 15^{a}	8 ± 27^{a}	-20 ± 30^{a}

[1] Values for delta abs are the mean and the SD x 10^{-3}.

[2] Means in the same row with the same superscript letter do not differ ($p>0.05$).

[3] Significant diet effects; $p<0.05$ for delta abs (0-28 days). DMCT and TMCT for delta abs are shown in Appendix 16. F-values for the statistical comparisons among diets are shown in Appendix 14.

[4] Values with the same superscript letter do not differ ($p>0.05$); classification based on DMCT in Appendix 16.

Table 14. Mean LDL Oxidation Rate (Day 0) and Percent Change (0-28 Days) for Experimental Diets

		Diet Group		
	CAN	LLNA	SNFLR	CONT
Mean Rate (ΔAbs/min)[1,2]				
M	105 ± 17	97 ± 14	109 ± 19	80 ± 30
F	102 ± 26	87 ± 10	99 ± 12	83 ± 15
% Change[2,3,4]				
M	2.38 [a]	-0.02 [a]	-1.42 [a]	23.65 [b]
F	5.42 [a]	8.68 [a]	-2.71 [b]	14.17 [a]

[1] Values for mean oxidation rate are mean and the SD x 10^{-4} at Day 0.

[2] There was a significant ($p<0.05$) diet x gender interaction for the change in LDL oxidation rate during the 0-28 day study period.

[3] Values expressed as a percent increase or decrease for the study period 0-28 days based on mean values at Day 0.

[4] Values in the same row with the same superscript letter do not differ ($p>0.05$). See linear contrasts in Appendix 12g.

Table 15. Mean LDL Oxidation Rate (Day 28) and Percent Change (28-56 Days) for Experimental Diets

	Diet Group			
	CAN	LLNA	SNFLR	CONT
Mean Rate (ΔAbs/min) [1,2]	102 ± 12	96 ± 14	101 ± 29	95 ± 14
% Change [3,4]	-1.70 [ab]	-6.50 [b]	10.26 [a]	-7.44 [b]

[1] All values for mean oxidation rate are mean and SD x 10^{-4} at Day 28.

[2] There was a significant diet effect ($p<0.05$) for the change in LDL oxidation rate during the 28-56 day study period.

[3] Values expressed as a percent increase or decrease for the study period 28-56 days based on the mean values at Day 28.

[4] Values with the same superscript letter do not differ ($p>0.05$); classification based on DMCT shown in Appendix 15.

Figure 9. Effect of Dietary Fat Source on LDL Oxidation Rate in Men and Women: 0-28 Days

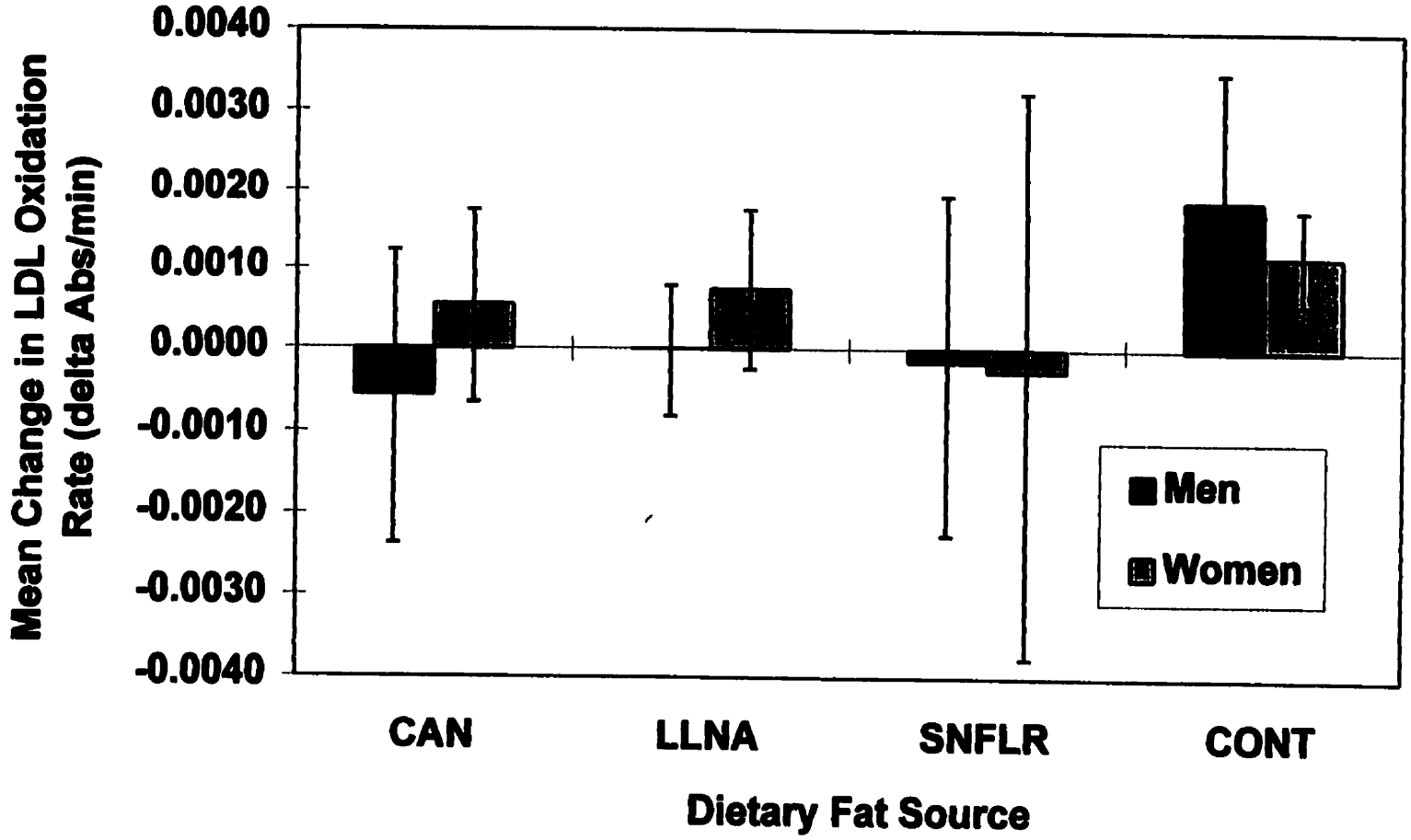
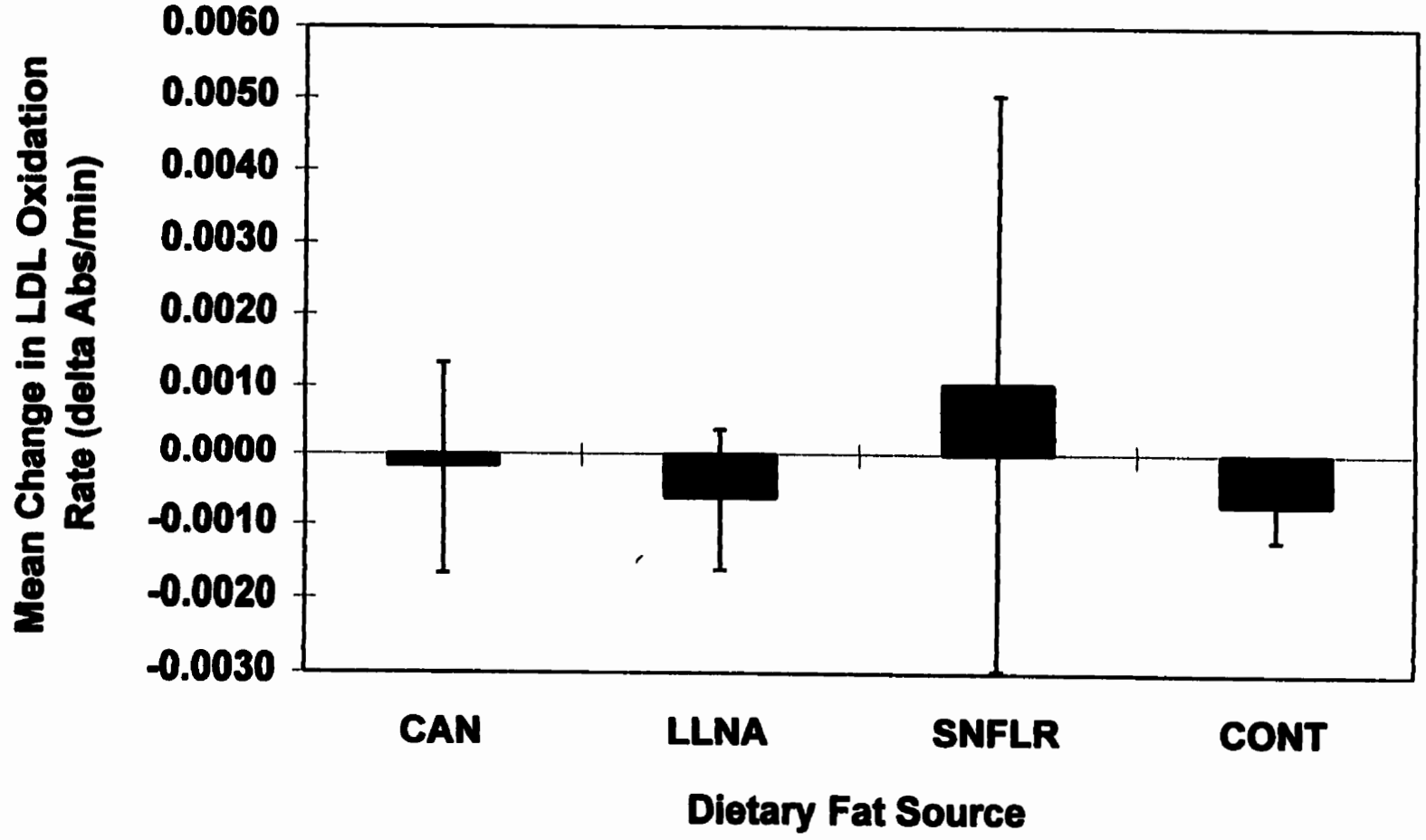


Figure 10. Effect of Dietary Fat Source on LDL Oxidation Rate: 28-56 Days



occurred on the SNFLR diet (10.3%). A small decrease (-1.7%) in LDL oxidation rate occurred on the CAN diet, while the LLNA and CONT diets resulted in greater decreases (-6.5 and -7.4%, respectively). However, no differences were found among the diets by the Tukey's multiple comparison test (Appendix 15); TMCT is a more conservative multiple comparison test than DMCT, although both DMCT and TMCT usually produce broadly comparable results (Hassard, 1991). The disparity between these two tests suggests that the diet differences are marginal. Hassard (1991) has suggested that the more severe standards used in TMCT can lead to subtle genuine differences being overlooked.

IV Effect of the Experimental Diets on Total Change in Absorbance (Delta Absorbance) in NIDDM Subjects

The experimental diets had no effect on the total change in absorbance (total amount of conjugated dienes produced) over the entire study period (0-56 days) or the last interval (28-56 days). However, a significant diet effect was found over the first month of the study (0-28 days). In general, it is assumed that the more total conjugated dienes formed, the greater the LDL oxidation. Mean total change in LDL absorbance and percent change for the experimental diets for the 0-28 day period are shown in Table 16 and Figure 11. DMCT and TMCT were performed to determine differences among diets (Appendix 16). DMCT showed the order of diet effects, beginning with the diet producing the largest amount of conjugated dienes (largest total change in absorbance) to be: CONT, 9.9% > SNFLR, 5.3% > CAN, 0.6% ≡ LLNA, -0.4%. The percent change in

Table 16. Change in Mean LDL Absorbance (Delta Abs at Day 0) During LDL Oxidation and Percent Change (0-28 Days) for Experimental Diets

	Diet Group			
	CAN	**LLNA**	**SNFLR**	**CONT**
Mean ΔAbs [1,2]	345 ± 25	330 ± 42	375 ± 54	343 ± 42
% Change [3,4]	0.55 [a]	-0.44 [a]	5.31 [ab]	9.93 [b]

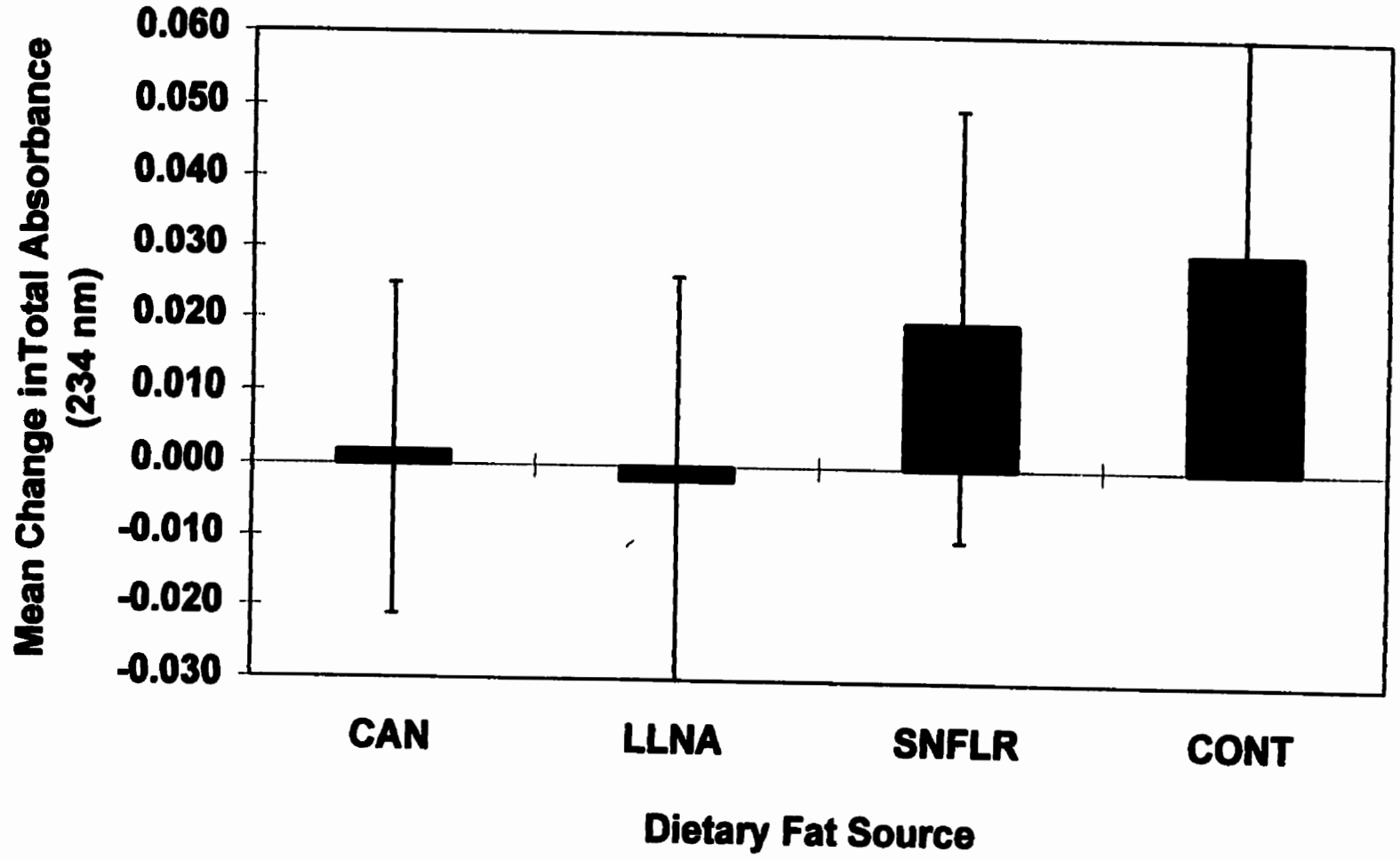
[1] All values for mean delta absorbance are mean and SD x 10^{-3} at Day 0.

[2] There was a significant diet effect ($p<0.05$) for the change in absorbance during the 0-28 day study period.

[3] Values expressed as a percent increase or decrease for the study period 0-28 days based on mean values at Day 0.

[4] Values with the same superscript letter do not differ ($p>0.05$); classification based on DMCT shown in Appendix 16.

Figure 11. Effect of Dietary Fat Source on Total Absorbance: 0-28 Days



total absorbance for the SNFLR diet was mid-way between the CONT and the CAN and LLNA diets. By contrast, there were no diet differences by the TMCT suggesting marginal effects of diet on lipid oxidation as measured by the formation of conjugated dienes.

V Effect of the Experimental Diets on LDL Fatty Acid Patterns in NIDDM Subjects

The LDL fatty acid data was statistically analyzed with the same model as the plasma total and lipoprotein cholesterol data. Changes in fatty acid composition of the LDL fraction in response to the experimental diets are reported as the mean change in percent fatty acids over the three study periods 0-56, 0-28, and 28-56 days (Tables 17b, 18, and 19b). Tables 17a and 19a present the mean percent fatty acid composition of the LDL fraction at Day 0 and Day 28, respectively.

There were no significant changes in the mean LDL fatty acid composition for myristic acid (C14:0), palmitic acid (C16:0), palmitoleic (C16:1), oleic acid (C18:1), linoleic acid (LA; C18:2n6), linolenic acid (LNA; C18:3n3), eicosatrienoic acid (C20:3), eicosapentaenoic acid (EPA; C20:5), docosapentaenoic acid (C22:5), and docosahexaenoic acid (DHA; C22:6) over the 0-56 day study period. Diet x gender interactions were found during this period for stearic acid (C18:0) and arachidonic acid (C20:4). From the linear contrasts shown in Appendix 12d and data in Table 20 and Figure 12, it is apparent that for women, the mean change in C18:0 levels differed significantly for the SNFLR diet (4.8%) compared to the CONT diet (-1.6%; $p<0.05$), whereas there were no significant differences in the mean change in C18:0 among the

Table 17a. Mean Percent Fatty Acid Composition of Lipid in the LDL Fraction at Day 0 [1]

	Diet Group			
	CAN	LLNA	SNFLR	CONT
Fatty Acid [2]				
14:0	1.10 ± 0.31	1.11 ± 0.26	1.02 ± 0.34	0.97 ± 0.61
16:0	22.30 ± 1.91	22.59 ± 1.80	22.16 ± 2.00	18.66 ± 2.83
16:1	3.44 ± 1.45	3.57 ± 1.00	2.98 ± 1.27	3.35 ± 1.43
18:0 [3]				
M	6.21 ± 0.57	5.87 ± 0.61	5.77 ± 0.66	5.92 ± 0.46
F	5.96 ± 0.78	5.92 ± 0.75	5.90 ± 0.97	5.99 ± 0.96
18:1	21.48 ± 2.07	23.14 ± 2.37	21.15 ± 1.95	24.14 ± 1.19
18:2	34.14 ± 3.73	32.96 ± 4.00	36.12 ± 5.51	33.66 ± 2.43
18:3	1.39 ± 0.28	1.26 ± 0.31	1.18 ± 0.17	1.26 ± 0.13
20:3	1.64 ± 0.52	1.40 ± 0.36	1.32 ± 0.37	1.24 ± 0.13
20:4 [3]				
M	6.81 ± 1.45	6.39 ± 1.86	7.19 ± 1.58	6.45 ± 2.52
F	6.82 ± 0.13	5.63 ± 1.33	5.84 ± 0.53	7.35 ± 1.81
20:5	0.63 ± 0.18	0.59 ± 0.24	0.75 ± 0.31	0.70 ± 0.16
22:5	0.22 ± 0.04	0.19 ± 0.05	0.34 ± 0.24	0.25 ±0.05
22:6	0.65 ± 0.16	0.74 ± 0.27	0.79 ± 0.39	0.86 ± 0.25

[1] All values are mean ± SD.

[2] Carbon chain length : number of double bonds.

[3] Significant diet x gender interactions were found for C18:0 and C20:4, therefore, the mean percent fatty acid levels for gender are given for these fatty acids.

Table 17b. Mean Change in Percent Fatty Acid Composition of LDL: 0-56 Days [1,2]

	Diet Group			
	CAN	LLNA	SNFLR	CONT
Fatty Acid [3]				
14:0	-0.07 ± 0.22 [a]	-0.03 ± 0.34 [a]	-0.08 ± 0.29 [a]	-0.11 ± 0.41 [a]
16:0	-0.35 ± 0.90 [a]	0.21 ± 1.48 [a]	-0.36 ± 1.77 [a]	-0.49 ± 2.40 [a]
16:1	-0.04 ± 0.55 [a]	0.084 ± 0.51 [a]	0.02 ± 0.77 [a]	-0.18 ± 0.72 [a]
18:0 [4]				
M	-0.29 ± 0.21	0.44 ± 0.49	0.09 ± 0.79	0.04 ± 0.65
F	0.11 ± 0.74	0.02 ± 0.45	0.28 ± 0.74	-0.10 ± 0.39
18:1	-0.38 ± 1.62 [a]	-0.27 ± 1.14 [a]	-0.06 ± 1.41 [a]	-1.04 ± 2.33 [a]
18:2	0.19 ± 2.67 [a]	0.18 ± 3.10 [a]	0.22 ± 3.81 [a]	0.46 ± 2.07 [a]
18:3	-0.06 ± 0.18 [a]	0.10 ± 0.45 [a]	0.15 ± 0.29 [a]	0.02 ± 0.33 [a]
20:3	0.05 ± 0.27 [a]	0.10 ± 0.40 [a]	0.003 ± 0.243 [a]	0.05 ± 0.18 [a]
20:4 [4]				
M	0.62 ± 0.61	1.33 ± 0.35	-0.15 ± 0.92	0.57 ± 0.98
F	0.54 ± 0.13	0.47 ± 1.11	0.19 ± 0.48	-0.41 ± 0.54
20:5	0.08 ± 0.11 [a]	-0.03 ± 0.18 [a]	-0.09 ± 0.23 [a]	0.00 ± 0.20 [a]
22:5	0.02 ± 0.03 [a]	0.03 ± 0.03 [a]	0.008 ± 0.047 [a]	0.001 ± 0.040 [a]
22:6	0.05 ± 0.08 [a]	-0.07 ± 0.22 [a]	-0.006 ± 0.109 [a]	-0.03 ± 0.20 [a]

[1] All values are mean ± SD. F-values and associated probabilities for the statistical comparison among experimental diets are shown in Appendix 17.

[2] Means in the same row with the same superscript letter do not differ ($p>0.05$).

[3] Carbon chain length : number of double bonds.

[4] Significant diet x gender interaction was found for C18:0 and C20:4. Linear contrasts, associated t-statistic, and probability values are shown in Appendix 12d and 12e.

Table 18. Mean Change in Percent Fatty Acid Composition of LDL: 0-28 Days [1,2]

	Diet Group			
	CAN	LLNA	SNFLR	CONT
Fatty Acid [3]				
14:0	-0.02 ± 0.31 [a]	-0.10 ± 0.29 [a]	-0.14 ± 0.24 [a]	-0.13 ± 0.58 [a]
16:0	-0.47 ± 1.22 [a]	-0.41 ± 1.30 [a]	-1.46 ± 1.59 [a]	-0.89 ± 2.54 [a]
16:1	-0.09 ± 0.67 [a]	-0.17 ± 0.61 [a]	-0.32 ± 0.57 [a]	-0.32 ± 1.05 [a]
18:0	0.11 ± 0.57 [a]	-0.06 ± 0.46 [a]	0.11 ± 0.80 [a]	0.10 ± 0.43 [a]
18:1	0.09 ± 1.49 [a]	-0.20 ± 1.14 [a]	0.33 ± 1.59 [a]	-0.68 ± 2.26 [a]
18:2	-0.72 ± 1.56 [a]	0.71 ± 2.30 [a]	1.20 ± 4.07 [a]	0.24 ± 2.50 [a]
18:3	0.10 ± 0.44 [a]	-0.08 ± 0.23 [a]	0.05 ± 0.38 [a]	0.08 ± 0.63 [a]
20:3	0.06 ± 0.19 [a]	0.02 ± 0.19 [a]	0.06 ± 0.11 [a]	0.04 ± 0.20 [a]
20:4 [4]				
M	0.98 ± 0.86	-0.23 ± 0.78	0.22 ± 0.76	1.10 ± 0.98
F	0.21 ± 1.13	0.90 ± 1.19	0.20 ± 0.67	-0.55 ± 0.29
20:5	0.09 ± 0.21 [a]	0.06 ± 0.02 [a]	-0.09 ± 0.29 [a]	0.02 ± 0.12 [a]
22:5	0.03 ± 0.05 [a]	0.03 ± 0.06 [a]	0.000 ± 0.05 [a]	-0.01 ± 0.05 [a]
22:6	0.10 ± 0.17 [a]	0.02 ± 0.20 [a]	0.08 ± 0.11 [a]	0.05 ± 0.19 [a]

[1] All values are mean ± SD. F-values and associated probabilities for the statistical comparison among experimental diets are shown in Appendix 18.

[2] Means in the same row with the same superscript letter do not differ ($p>0.05$).

[3] Carbon chain length : number of double bonds.

[4] Significant diet x gender interaction for C20:4. Linear contrasts, associated t-statistic, and probability values are shown in Appendix 12f.

Table 19a. Mean Percent Fatty Acid Composition of Lipid in the LDL Fraction at Day 28 [1]

	Diet Group			
	CAN	LLNA	SNFLR	CONT
Fatty Acid [2]				
14:0	1.08 ± 0.27	1.01 ± 0.30	0.88 ± 0.28	0.84 ± 0.26
16:0	21.81 ± 1.57	22.19 ± 1.85	22.71 ± 2.16	20.98 ± 1.74
16:1	3.35 ± 1.25	3.40 ± 1.18	2.65 ± 0.99	3.02 ± 0.90
18:0				
M	6.42 ± 0.70	5.79 ± 0.18	6.12 ± 0.41	5.95 ± 0.40
F	5.98 ± 0.37	5.88 ± 0.62	5.81 ± 0.37	6.14 ± 1.22
18:1	21.57 ± 1.45	22.95 ± 2.05	21.48 ± 1.78	24.22 ± 1.99
18:2	33.55 ± 3.85	33.67 ± 4.15	37.32 ± 4.92	33.38 ± 4.00
18:3	1.49 ± 0.41	1.18 ± 0.26	1.23 ± 0.35	1.34 ± 0.69
20:3	1.70 ± 0.39	1.42 ± 0.31	1.37 ± 0.37	1.27 ± 0.26
20:4				
M	7.79 ± 0.86	6.17 ± 1.21	7.41 ± 1.32	7.55 ± 1.94
F	7.03 ± 1.60	6.53 ± 1.53	6.04 ± 0.71	6.80 ± 1.85
20:5	0.73 ± 0.16	0.65 ± 0.20	0.66 ± 0.24	0.72 ± 0.19
22:5	0.25 ± 0.06	0.22 ± 0.05	0.34 ± 0.25	0.24 ± 0.07
22:6	0.75 ± 0.23	0.76 ± 0.17	0.89 ± 0.37	0.91 ± 0.35

[1] All values are mean ± SD.

[2] Carbon chain length : number of double bonds.

Table 19b. Mean Change in Percent Fatty Acid Composition of LDL: 28-56 Days [1,2]

	Diet Group			
	CAN	LLNA	SNFLR	CONT
Fatty Acid [3]				
14:0	-0.05 ± 0.19 [a]	0.12 ± 0.22 [a]	0.06 ± 0.18 [a]	0.02 ± 0.27 [a]
16:0	0.12 ± 0.92 [a]	0.62 ± 1.08 [a]	1.10 ± 1.41 [a]	0.40 ± 1.48 [a]
16:1	0.05 ± 0.74 [a]	0.23 ± 0.43 [a]	0.35 ± 0.53 [a]	0.14 ± 0.42 [a]
18:0	-0.20 ± 0.46 [a]	0.11 ± 0.47 [a]	0.09 ± 0.76 [a]	-0.13 ± 0.59 [a]
18:1	-0.47 ± 1.76 [a]	0.24 ± 1.78 [a]	-0.39 ± 0.69 [a]	-0.36 ± 1.59 [a]
18:2	0.91 ± 2.63 [a]	-0.88 ± 2.33 [a]	-0.98 ± 2.28 [a]	0.08 ± 2.62 [a]
18:3	-0.16 ± 0.47 [a]	0.13 ± 0.34 [a]	0.10 ± 0.48 [a]	-0.06 ± 0.60 [a]
20:3	-0.02 ± 0.20 [a]	0.03 ± 0.36 [a]	-0.05 ± 0.25 [a]	0.01 ± 0.11 [a]
20:4	-0.01 ± 1.00 [a]	-0.44 ± 0.49 [a]	-0.17 ± 0.34 [a]	-0.16 ± 0.88 [a]
20:5	-0.01 ± 0.22 [a]	-0.07 ± 0.14 [a]	0.01 ± 0.24 [a]	-0.02 ± 0.27 [a]
22:5	-0.01 ± 0.06 [a]	-0.01 ± 0.06 [a]	0.001 ± 0.05 [a]	0.01 ± 0.05 [a]
22:6	-0.04 ± 0.18 [a]	-0.08 ± 0.09 [a]	-0.09 ± 0.16 [a]	-0.08 ± 0.21 [a]

[1] All values are mean ± SD. F-values and associated probabilities for the statistical comparison among experimental diets are shown in Appendix 19.

[2] Means in the same row with the same superscript letter do not differ ($p>0.05$).

[3] Carbon chain length : number of double bonds.

Table 20. Mean LDL C18:0 Fatty Acid Levels at Day 0 and Percent Change (0-56 Days) for Experimental Diets

	Diet Group			
	CAN	LLNA	SNFLR	CONT
Mean [1,2]				
M	6.21 ± 0.57	5.87 ± 0.61	5.77 ± 0.66	5.93 ± 0.46
F	5.96 ± 0.78	5.92 ± 0.75	5.90 ± 0.97	5.99 ± 0.96
% Change [3,4]				
M	-4.71 [a]	7.41 [b]	1.61 [ab]	0.68 [ab]
F	1.84 [ab]	0.30 [ab]	4.75 [b]	-1.64 [a]

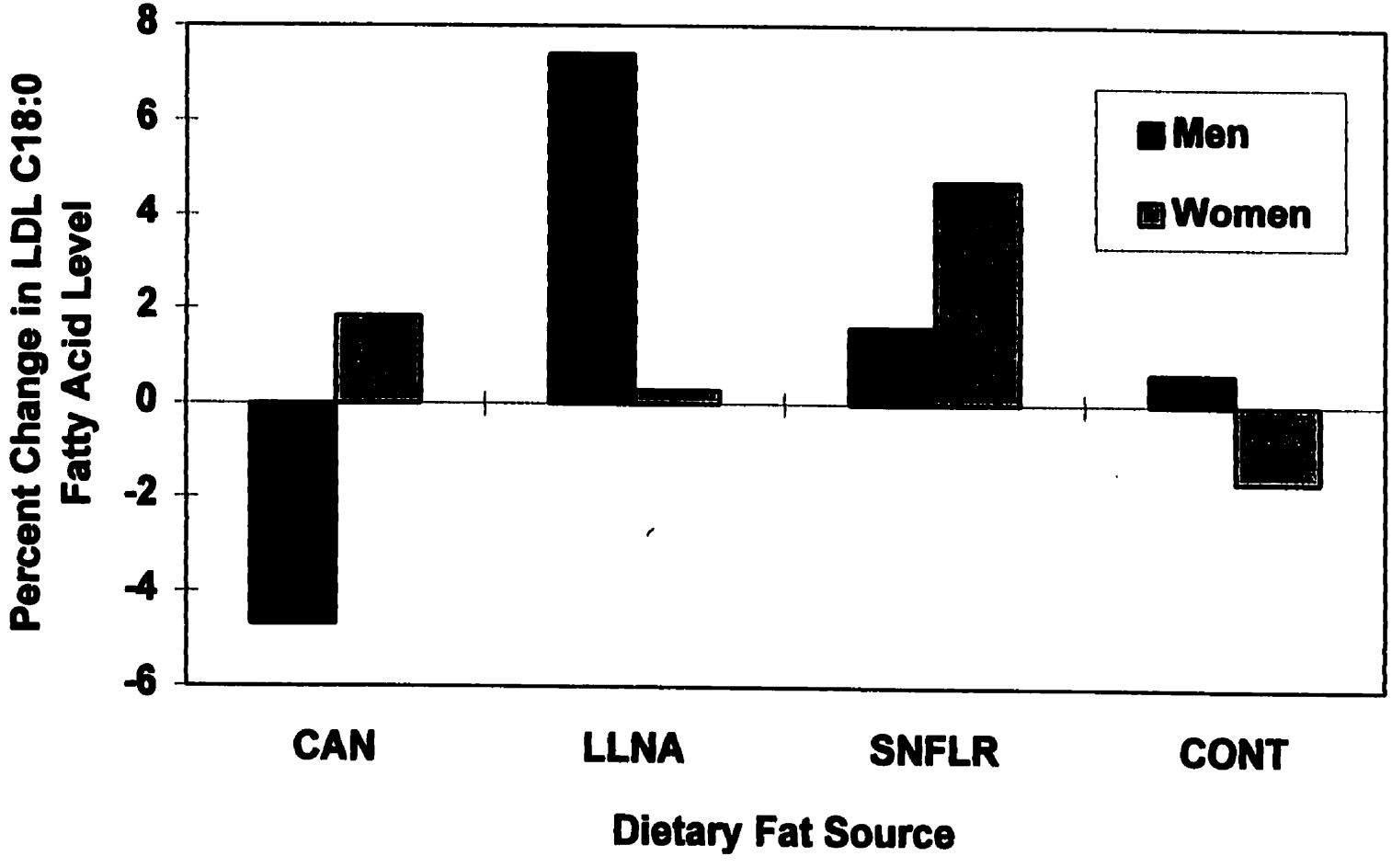
[1] All values for mean percent C18:0 levels are mean ± SD at Day 0.

[2] There was a significant ($p<0.05$) diet x gender interaction for LDL C18:0 levels during the 0-56 day study period.

[3] Values expressed as a percent increase or decrease for the study period 0-56 days based on mean values at Day 0.

[4] Values with the same superscript letter do not differ ($p>0.05$); classification based on linear contrast in Appendix 12d.

Figure 12. Effect of Dietary Fat Source on LDL C18:0 Levels in Men and Women: 0-56 Days



other diets. For men, LDL C18:0 content decreased 4.7% on the CAN diet compared to a 7.4% increase on the LLNA diet ($p<0.01$), whereas no differences occurred with the other diets. Opposite diet effects of interest for men and women included a slight increase in C18:0 levels for women on the CAN diet (1.8%) compared to a decrease on the CONT diet (-1.6%), and an opposite response in this fatty acid for men on the same diets (-4.7% and 0.7% for CAN and CONT, respectively). C18:0 levels remained constant for women on the LLNA diet whereas levels for men increased (7.4%). When CAN and LLNA diets are compared, women show a trend toward increased C18:0 levels, unlike men who show a significant ($p<0.01$) decrease on the CAN compared to an increase on the LLNA diet.

Appendix 12e, Table 21, and Figure 13 show that for women on the CAN and LLNA diets there were mean increases in LDL C20:4 levels (7.9% and 8.4% for CAN and LLNA, respectively) whereas there was a decrease on the CONT (-5.6%) during the study. These differences were statistically significant (Appendix 12e). The SNFLR diet also resulted in an increase in mean LDL C20:4 level but the change did not differ from any of the other dietary regimen. For men, it is difficult to interpret these findings due to the unbalanced LLNA group (contained only 2 subjects). Nonetheless, there was a decrease (20.7%) in LDL 20:4 level on the LLNA diet compared to increases of 8.8% on the CONT diet and 9.1% on the CAN diet. As a result, the changes on the LLNA and the CONT and CAN diets differed significantly ($p<0.0006$ and $p<0.005$, respectively; Appendix 12e). There was also a slight decrease in mean C20:4 level on the SNFLR diet for men (2.1%) whereas there was a slight increase for women (3.3%). No significant diet effects were found for any other fatty acids.

Table 21. Mean LDL C20:4 Fatty Acid Level at Day 0 and Percent Change (0-56 Days) for Experimental Diets

	Diet Group			
	CAN	LLNA	SNFLR	CONT
Mean [1,2]				
M	6.81 ± 1.45	6.39 ± 1.86	7.19 ± 1.58	6.45 ± 2.52
F	6.82 ± 1.30	5.63 ± 1.33	5.84 ± 0.53	7.35 ± 1.81
% Change [3,4]				
M	9.13 [a]	-20.73 [b]	-2.12 [ab]	8.76 [a]
F	7.92 [a]	8.35 [a]	3.32 [ab]	-5.61 [b]

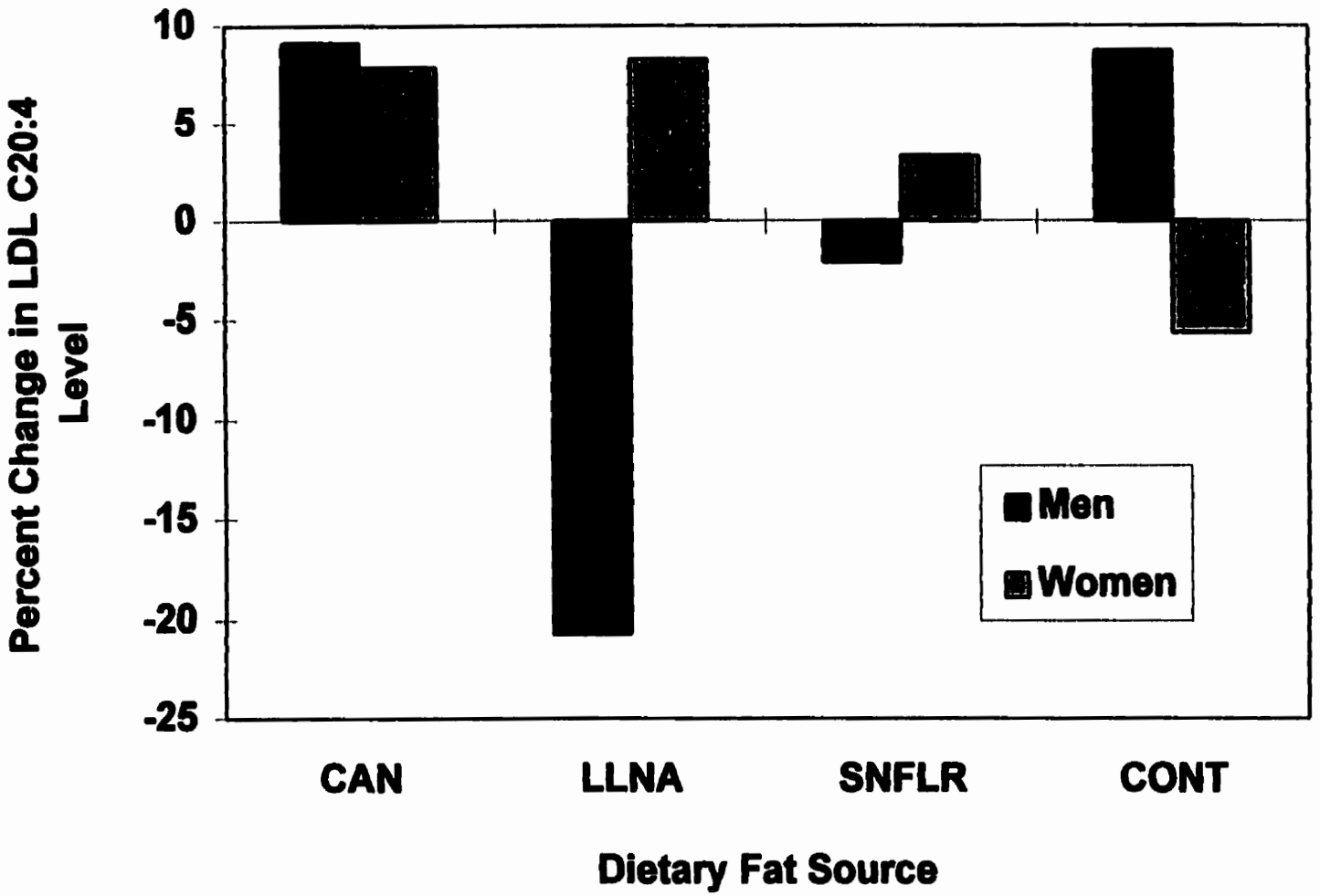
[1] All values for mean percent C20:4 levels are mean ± SD at Day 0.

[2] There was a significant ($p<0.05$) diet x gender interaction for LDL C20:4:0 levels during the 0-56 day study period.

[3] Values expressed as a percent increase or decrease for the study period 0-56 days based on the mean values at Day 0.

[4] Values sharing the same superscript letter do not differ ($p>0.05$); classification based on linear contrast in Appendix 12e.

Figure 13. Effect of Dietary Fat Source on LDL C20:4 Levels in Men and Women: 0-56 Days



During the study period 0-28 days (Table 22 and Figure 14) there was a significant diet x gender interaction for the change in LDL C20:4 levels. C20:4 levels for women increased 13.8% on the LLNA diet ($p<0.01$) whereas for men on the LLNA diet C20:4 levels decreased 3.7% ($p<0.03$). By contrast, C20:4 level for men increased on the CONT diet (14.5%) whereas it decreased for women (8.0%). There was also a significant difference in the changes on the CAN and LLNA diets; C20:4 levels increased 12.5% for men on the CAN diet whereas on the LLNA diet, levels decreased 3.7% ($p<0.04$). The changes on the CAN (3.0%) and SNFLR (3.4%) diets for women did not differ significantly from either the LLNA (13.8%) or the CONT (-8.0%) diets.

VI Effect of Experimental Diets on LDL Particle Size Parameters: Cor/Sur Ratio, Radius, and Diameter

The LDL particle size parameters, including LDL cor/sur ratio, radius, and diameter, were calculated according to the equations of Van Heek and Zilversmit (1991) which are summarized in Appendix 9. Statistical analysis of the LDL size characteristics were carried out in the same way as described for plasma total and lipoprotein cholesterol data. Mean change in these parameters in response to the experimental diets are reported as the mean change over the study periods 0-56, 0-28, and 28-56 days (Tables 23 and 24).

There were no differences due to diet in the change in LDL parameters: namely cor/sur ratio, radius, or diameter during any of the study periods. However, significant gender differences were found for all parameters over the 0-56 day study period (Table 25 and Figure 15); LDL cor/sur ratio, radius, and diameter increased appreciably more for

men than women. Percent change in LDL cor/sur ratio, radius, and diameter were 22.0%, 22.0%, and 16.3% for men, and 0.1%, 9.7%, and 7.3% for women. However, large variability was found in these parameters.

Table 22. Mean LDL C20:4 Fatty Acid Levels at Day 0 and Percent Change (0-28 Days) for Experimental Diets

	Diet Group			
	CAN	LLNA	SNFLR	CONT
Mean [1,2]				
M	7.79 ± 0.86	6.17 ± 1.21	7.41 ± 1.32	7.55 ± 1.94
F	7.03 ± 1.60	6.53 ±1.53	6.04 ± 0.71	6.80 ± 1.85
% Change [3,4]				
M	12.54 [a]	-3.65 [b]	2.97 [ab]	14.51 [a]
F	2.96 [ab]	13.84 [a]	3.38 [ab]	-8.02 [b]

[1] All values for mean C20:4 levels are mean percent ± SD at Day 0.

[2] There was a significant ($p<0.05$) diet x gender interaction for LDL C20:4:0 levels during the 0-28 day study period.

[3] Values expressed as a percent increase or decrease for the study period 0-28 days based on the mean values at Day 0.

[4] Values in the same row with the same superscript letter do not differ ($p>0.05$); classification based on linear contrasts in Appendix 12f.

Table 23. Mean Change in LDL Particle Size Parameters: 0-56 Days [1,2]

	Parameters		
Variables	**Cor/sur**	**Radius**	**Diameter**
CAN	0.18 ± 0.17 [a]	11.63 ± 10.78 [a]	23.20 ± 21.60 [a]
LLNA	0.12 ± 0.11 [a]	7.49 ± 7.25 [a]	14.88 ± 14.67 [a]
SNFLR	0.15 ± 0.14 [a]	9.77 ± 8.94 [a]	19.56 ± 17.74 [a]
CONT	0.14 ± 0.17 [a]	8.74 ± 11.25 [a]	17.67 ± 22.58 [a]
Males [3]	0.21 ± 0.16	13.41 ± 10.39	26.81 ± 20.85
Females	0.10 ± 0.12	6.42 ± 7.59	12.85 ± 15.18

[1] All values are mean ± SD. F-values and associated probabilities for the statistical comparison among experimental diets are shown in Appendix 20.

[2] Means in the same column with the same superscript letter do not differ ($p>0.05$).

[3] Significant gender differences ($p<0.04$) for all three LDL particle size parameters including the cor/sur volume ratio, radius, and diameter.

Table 24. Mean Change in LDL Particle Size Parameters: 0-28 and 28-56 Days [1,2]

	Diet Group			
	CAN	LLNA	SNFLR	CONT
Parameters				
0-28 Days				
Cor/sur	0.12 ± 0.30 [a]	0.09 ± 0.13 [a]	0.16 ± 0.12 [a]	0.26 ± 0.23 [a]
Radius	7.97 ± 19.51 [a]	5.56 ± 8.16 [a]	9.97 ± 7.74 [a]	17.11 ± 14.79 [a]
Diameter	15.90 ± 39.03 [a]	11.00 ± 16.14 [a]	19.89 ± 15.62 [a]	34.43 ± 29.51 [a]
28-56 Days				
Cor/sur	0.06 ± 0.22 [a]	0.02 ± 0.11 [a]	0.00 ± 0.14 [a]	-0.11 ± 0.24 [a]
Radius	3.66 ± 13.91 [a]	1.51 ± 7.13 [a]	-0.20 ± 8.96 [a]	7.19 ± 15.59 [a]
Diameter	7.30 ± 27.67 [a]	3.00 ± 14.44 [a]	-0.33 ± 17.83 [a]	-14.43 ± 30.94 [a]

[1] All values are mean ± SD. F-values and associated probabilities for the statistical comparison among experimental diets are shown in Appendix 20.

[2] Means in the same row with the same superscript letter do not differ ($p>0.05$).

Table 25. Mean LDL Particle Size Parameter Levels (Day 0) and Percent Change (0-56 Days) for Males and Females

	LDL Particle Size Parameters (0-56 days)		
	Cor/Sur	Radius	Diameter
Mean [1,2]			
M	0.95 ± 0.18	60.94 ± 11.55	164.88 ± 23.15
F	1.03 ± 0.14	66.35 ± 9.05	175.70 ± 18.09
% Change [3]			
M	22.03	22.00	16.26
F	0.10	9.67	7.31

[1] All values for mean LDL particles size parameter levels are mean ± SD at Day 0.

[2] There were significant gender differences ($p<0.05$) for the LDL particle size parameters cor/sur ratio, radius, and diameter during the 0-56 day study period.

[3] Values expressed as a percent increase or decrease for the study period 0-56 days based on the mean values at Day 0.

Figure 14. Effect of Dietary Fat Source on LDL C20:4 Levels in Men and Women: 0-28 Days

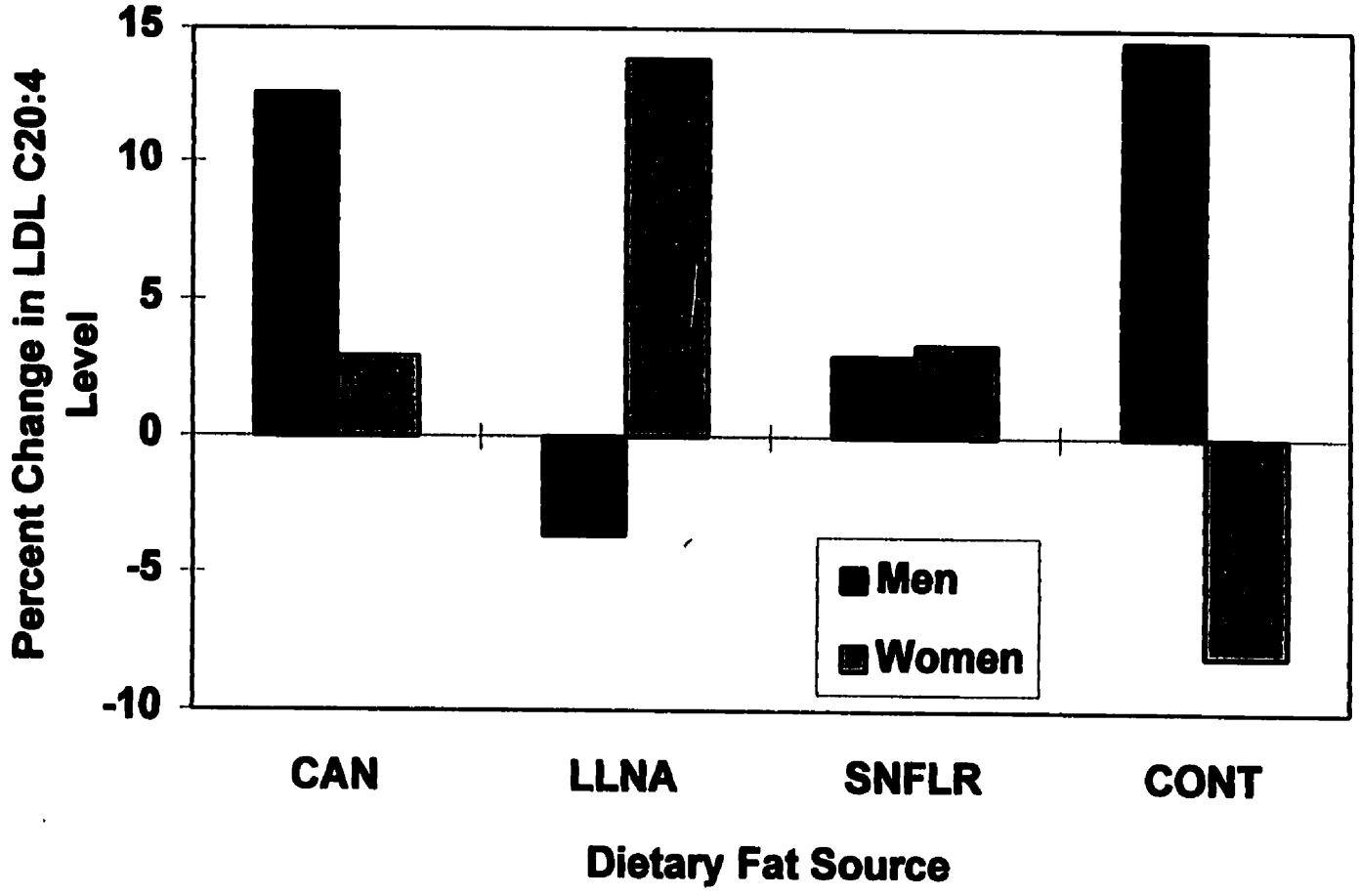
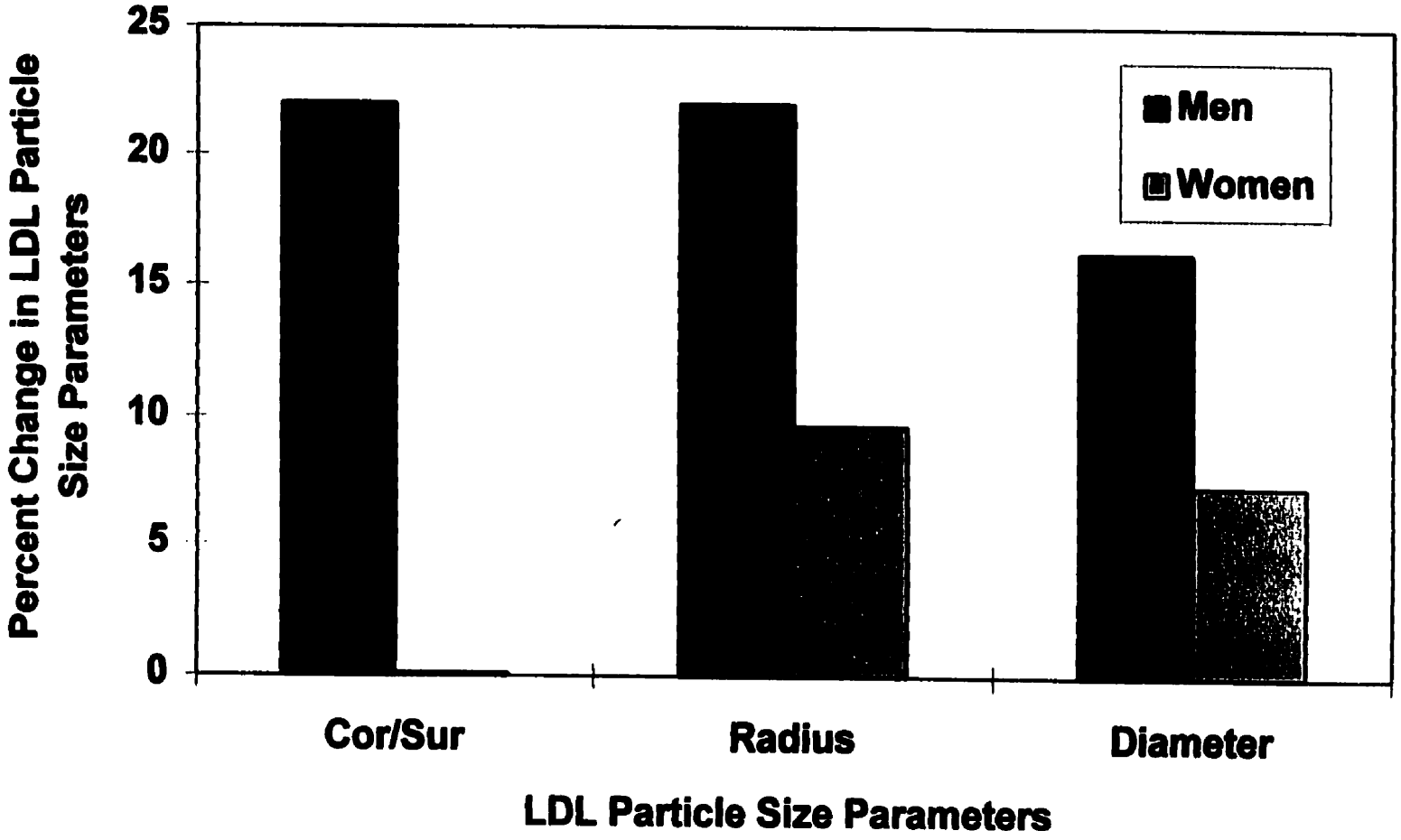


Figure 15. Differences in LDL Particle Size Parameters in Men and Women: 0-56 Days



DISCUSSION

I Subject Body Fat Distribution and Dietary Data

In order to assess health risk from obesity, body mass index (BMI; Kg/m^2) was calculated for each subject. Minor, insignificant differences in BMI were found among each dietary group and/or gender category. BMI's of 21-26, 22-27, 23-28, and 24-29 in individuals between the ages 35-44, 45-54, 55-65, and 65^+, respectively, are interpreted as acceptable with respect to health risk from obesity (Zeman, 1991). These ranges in BMI represent acceptable adjustments for age. The average age of the participants in the present study was 54 years. The average BMI in the present study was 31 ± 7.1; 25 subjects (10 men and 15 women) were above the acceptable BMI range for their age, while 11 subjects (5 men and 6 women) fell within the normal range for their age. Energy intake did not differ among dietary groups (Table 1) although the large variation in energy intake within and among dietary groups could have masked any real differences. Similarly, protein intake did not differ significantly among the dietary groups. However, men had significantly higher protein intakes than women which was probably due to the trend towards higher energy intake for men. CHO intake showed no significant differences among dietary groups. However, for women a higher proportion of daily energy was derived from CHO than for men, most likely due to the lower levels of intake of animal product intake and their substitution by high-CHO foods such as breads, pastas, and baked goods.

II Glycosylated Hemoglobin, Hb_{A1C}

There were no significant differences in GlyHb levels among the dietary fat groups during any of study periods (Table 2). However, men had a significantly smaller change in GlyHb levels than women over the 56 day study period. There was a decrease in GlyHb levels for men during this period regardless of dietary fat group, whereas GlyHb levels tended to increase for women (8.4 % decrease for men versus 3.4% increase for women). Glycosylation of LDL due to prolonged hyperglycemia has been shown to be related to the oxidizability of LDL (Hunt et al, 1990).

III Dietary Fat Intake

There were no differences in percent total fat consumed among dietary groups (Table 1). All subjects were counseled to consume a low-fat diet, that is, 30% of total energy from fat. All dietary groups averaged slightly over 30% of total energy as fat and there was wide variability in fat intake in all groups. The average percentage of total energy from fat for all subjects combined was 33.8 ± 2.4 % which is in the low-fat diet category. Spread and oil intake was very similar among experimental groups; the percent of total daily fat from dietary spread/oil was 13.4 ± 7.8% (i.e. 4.5% of total energy).

The intake of saturated and monounsaturated fatty acids did not differ significantly among the dietary groups (Table 1) even though the spread and oil for the CAN and LLNA groups contained higher levels of OA than the spread and oil for the SNFLR group (Appendices 3 and 4). The reason for the absence of any differences may be due to the already low amount of fat consumed daily, thus limiting any significant

change in fatty acid composition of the diets. However, PUFA intake did differ significantly among dietary fat groups. The CAN and LLNA groups had similar PUFA intakes which were much higher than for the CONT diet. The highest level of PUFA intake, however, was on the SNFLR diet which is consistent with the higher levels of PUFA in this spread/oil. The combined level of C18:2 and C18:3 in the spread was 47.5, 22.4, and 21.3 % for SNFLR, CAN, and LLNA dietary groups, respectively. Therefore, there was more than double the amount of PUFA (per tsp) in the SNFLR spread than in the CAN and LLNA spreads. However, the CAN, LLNA, and SNFLR groups did not differ in PUFA intake. The PUFA intake by the SNFLR group was significantly greater than the intake by the CONT group only. The spread for the CAN group contained 7.7% LNA, whereas the LLNA and SNFLR spread contained 2.2 and 0.4% of fatty acids as LNA, respectively (Appendix 3). Hence, the fat supplied by the LLNA diet was only 5% lower in LNA than that supplied by the CAN diet. Overall, there was very little energy obtained from the fats supplied to the subjects. On a total energy basis, the average energy intake from the experimental spreads was 4.8%, 5.4%, 4.5%, and 6.7% of energy for the CAN, LLNA, SNFLR, and CONT groups, respectively. The average intake of LNA by the CAN group was 6.5 Kcal/day, whereas the intakes by LLNA and SNFLR groups were 1.9 and 0.3 Kcal/day. Because the fatty acid composition of the diets of the CONT group is unknown, a comparison with this group could not be made. One drawback to the low-fat restriction of the study was the limited room for manipulation of the fat component of the diet among the experimental groups.

Average cholesterol intake was similar among all dietary fat groups

(263 ± 121 mg/d) but it varied widely among subjects and within the 3-day food records for each subject.

V Plasma Total and Lipoprotein Cholesterol Concentrations

(a) Total and Low Density Lipoprotein Cholesterol

Serum TC and LDL-C levels are commonly within the normal range in NIDDM individuals with reasonable glycemic control (Dimitriadis et al, 1995; Ronnemaa et al, 1989). In the present study, TC concentrations (5.44-5.64 mmol/L) were only slightly above the threshold of the normal range (2.95-5.20 mmol/L) in each dietary fat group at the beginning of the study (Table 4). Overall, 22 subjects (5 men and 15 women) were above the TC threshold level while 14 subjects (9 men and 5 women) were within the normal TC range. However, there was substantial variation within the dietary groups.

There were no differences in LDL-C concentrations among the dietary groups (Table 4). For men, average LDL-C concentrations were within normal levels (1.68-3.40 mmol/L) for the CAN, LLNA, and CONT groups; the average concentration for the SNFLR group was slightly above the threshold of the normal range for LDL-C concentration (3.57 ± 1.16 mmol/L) with three of the four male SNFLR subjects above threshold level. For women, the CAN and LLNA groups were within normal LDL-C concentration range (3.11 ± 1.08 and 3.17 ±0.57 mmol/L, respectively), whereas the SNFLR and CONT groups were slightly above the normal range (3.72 ± 0.86 and

3.65 ± 1.18 mmol/L, respectively). In the SNFLR group, three women were over the LDL-C threshold and two were within normal range. In the CONT group, two women were over the LDL-C threshold and three were within normal range.

The major emphasis of the study was to determine whether differences in fatty acid composition in the diets of NIDDM patients, namely differences in LNA or PUFA level, would produce significant differences in their lipoprotein metabolism, LDL fatty acid composition, and/or susceptibility of the LDL to oxidation. The absence of any effects of diet on TC for any study period (Table 5-8) is not surprising because fatty acid manipulation of the diet was undertaken in the context of a low-fat, high-CHO diet. Subjects who are already consuming low levels of total fat and saturated fat are already at a relatively low percentage of daily total energy intake from fat. It was a great challenge to change the fatty acid content of the diet in this highly restrictive environment.

The same reasoning can be applied to the changes in mean LDL-C concentrations during the study, although, the diet x gender interaction during the 0-56 day study period is difficult to explain given that MUFA and PUFA have been shown to be equally effective at reducing TC and LDL-C concentrations (Mattson and Grundy, 1985; Mensink and Katan, 1989). The data for the men on the study followed expected patterns; similar decreases occurred in mean LDL-C concentration on the CAN, LLNA, and SNFLR diets. LDL-C data for women over the 56 day study period are confusing. For women, similar decreases in mean LDL-C occurred on the LLNA, SNFLR, and CONT diets. However, there was no change in LDL-C concentrations for the CAN group. It was expected that the CAN group would follow the same pattern as the LLNA

and SNFLR groups because the diets differed only in MUFA and PUFA content and these dietary fatty acids usually produce equivalent effects on LDL-C concentrations. The CAN spread contained higher levels of LNA than the LLNA and SNFLR spreads. However, the 3-day food records showed no significant differences in PUFA intake among the CAN, LLNA, and SNFLR groups and Chan et al (1991) and Kestin et al (1990) found LNA equivalent to oleic acid and LA in lowering serum LDL-C levels in normal individuals.

(b) High Density Lipoprotein Cholesterol

HDL-C concentrations, at day 0, were within the normal range (0.9-2.0 mmol/L) for all subjects and did not appear to be depressed. There was moderate within group variability. The diet x gender interaction (0-28 days) may be partly explained by reports that the effect of diet on HDL-C concentrations may differ for men and women (Crouse, 1980; Mensink and Katan, 1987). Data from the present study partially support these earlier findings in that HDL-C concentrations did not change significantly for men on any of the experimental diets. For women, the CAN, SNFLR, and CONT diets produced no change in HDL-C, whereas the LLNA diet resulted in a significant increase in HDL-C. However, there is no obvious explanation for the differences in response to diet among the women subjects.

(c) Total : HDL Cholesterol Ratio

The ratio of TC to HDL-C has been reported to be the best biochemical predictor of myocardial infarction (Campos et al, 1992; Stampter et al, 1991). Valsta et al (1992) reported a decrease in TC:HDL-C ratio when MUFA and PUFA enriched diets replaced SFA in a conventional fat diet (38% total energy as fat). During the study period 28-56 days, in the present study, there was a significant diet effect on the TC:HDL-C ratio (Table 8). On CAN, LLNA, and SNFLR diets, the ratio decreased only slightly, whereas, on the CONT diet, there was a significant decrease in TC:HDL-C ratio. However, there was high within group variability during this period. The diet x gender interaction observed during the study period 0-28 days is unexplainable; the change in ratio followed opposite trends with gender on each of the diets. Castelli et al (1986) compared effects of a high-CHO diet with a high-MUFA diet in NIDDM and reported a significant improvement in the ratio of TC: HDL-C on the high-MUFA diet as compared with the high-CHO diet and an overall reduction of coronary risk.

(d) Triglycerides

In the present study, subject TAG concentrations were within the normal range (0.3-2.3 mmol/L) for the experimental groups on the CAN, LLNA, and SNFLR diets. The mean serum TAG concentration for the CONT group was only slightly above the threshold of the normal range; three women and one man of nine subjects in total were above the threshold TAG level. Overall, 10 subjects (4 men and 6 women) were above

the TAG threshold level and 26 subjects (12 men and 14 women) were within normal range. There was high variability in TAG concentrations for all diet groups.

Serum triglyceride concentrations usually correlate with glycosylated hemoglobin levels (Lahdenpera et al, 1996). Over the 0-56 day study period, there was a significant difference in mean GlyHb levels between men and women; for women GlyHb levels increased, whereas they decreased for men. This gender effect may have influenced the gender differences in serum TAG concentrations observed during this period. However, the established relationship of gender and TAG is opposite to the gender effect on GlyHb found in the present study; TAG concentrations decreased for women and increased for men whereas GlyHb levels increased for women and decreased for men. Valsta et al (1992) reported that differences in total TAG concentrations, were pronounced for women but non-significant for men when MUFA and PUFA diets were compared. Conversely, in the same study, the effects of the MUFA and PUFA diets on LDL and HDL were more apparent in men than women. Both MUFA and PUFA diets were equally effective in producing the above effects.

TAG concentration is the major determinant of LDL particle size (Lahdenpera et al, 1996). This effect was demonstrated in the present study in that mean change in TAG concentrations and LDL particle diameter (size) increased significantly in men and decreased in women which coincided with the changes in mean serum TAG concentrations.

V LDL Oxidation Rate and Total (Delta) Absorbance

The increased risk of atherosclerotic disease in NIDDM patients may be due to enhanced foam cell formation as a result of increased susceptibility of LDL to oxidative modification (Dimitriadis et al, 1995). As well, glycation of LDL-C has been shown to be related to its susceptibility to oxidization (Hunt et al, 1990). The present study attempted to compare fatty acid composition of LDL to its oxidative susceptibility. Lipid peroxidation is a complex process and *in vitro* methods of measuring oxidation assess different stages of lipid peroxidation. In the present study, LDL oxidation was measured by the formation of conjugated dienes in the presence of copper ions. Both rate of LDL oxidation and the total absorbance, that is, the total amount of conjugated dienes formed, were measured.

(a) LDL Oxidation Rate

There were negligible differences among dietary fat groups in LDL oxidation rate for the study period 0-56 days (Table 13a). However, during the first month of the study (0-28 days), a significant diet x gender effect was observed. During the second month (28-56 days), significant differences among the experimental diets were observed. LDL oxidation rate increased equally for women on the CONT, CAN, and LLNA diets during the period 0-28 days. This increase differed significantly from the slight decrease in LDL oxidation rate for women on the SNFLR diet during this same period even though there was greater variation in the rate of oxidation in the SNFLR group. Whether the decrease in LDL oxidation for the SNFLR group is physiologically significant is not clear at this

time. It seems that this data contradicts other evidence supporting the increased oxidation of LDL of subjects fed diets containing higher levels of PUFA. There is no obvious explanation for these observations. Reaven et al (1991) found that the extent of oxidation of LDL was influenced by its concentration of linoleic acid. In the present study, linoleic acid concentration of LDL did not change. An increase in LDL oxidation rate for men on the CONT diet occurred during the 0-28 day period and the increase tended to be greater than for the women on this diet. The CAN, LLNA, and SNFLR diets had similar effects on LDL oxidation rate in men. No studies to date have determined the effects of changes in dietary fatty acid intake on a low-fat, high-CHO diet on LDL oxidation in NIDDM. Similarly, there are no studies on the effect of gender on LDL oxidation in NIDDM patients. However, the importance and meaning of the diet x gender interaction in the present study is unknown.

The effect of the experimental diets on LDL oxidation during the second month of the study (28-56 days) may be more helpful in terms of assessing the importance of fatty acid unsaturation and rate of oxidation of the lipid in LDL. In this case, there was a similar decrease in LDL oxidation rate on the CONT and LLNA diets whereas there was an increase on the SNFLR diet. There was no change in oxidation rate on the CAN diet and the oxidation rate on this diet did not differ from that on the CONT, LLNA, or SNFLR diets. These findings tend to agree with those of other researchers who concluded that the more highly unsaturated the fat component of the diet, the higher the incorporation of these fatty acids into LDL and hence, the more susceptible the lipid in LDL will be to oxidization (Reaven, 1988). However, there were no differences in LDL

fatty acid composition among diets for this period of the study. The significant increase in the rate of LDL oxidation observed for the SNFLR group was expected because of the higher levels of PUFA intake in the diet. Whether gender differences in LDL oxidation exist is unknown. The specific effects of NIDDM on LDL oxidation is also unknown at this time. Evidence points to higher overall LDL oxidative susceptibility making it an important factor in the increased risk of CHD seen in these individuals and the pathogenesis of NIDDM. An important consideration for this effect may be related to hyperglycemia and increased production of glycosylated end-products which increase oxidative stress in the NIDDM individual.

(b) Total Conjugated Diene Production

The overall change in absorbance reflects the amount of conjugated dienes formed and hence, the amount of LDL oxidation (Kleinveld et al, 1993). Dimitriadis et al (1995) found that significantly higher total conjugated diene levels were produced with LDL from NIDDM than with LDL from control subjects. As well, NIDDM subjects were found to have significantly higher rates of diene formation than controls. In the present study, there were no differences among the experimental diets in total diene production during the study period 0-56 days or the second month of the study (28-56 days; Table 13b). However, a significant diet effect was found for the first month of the study (0-28 days) which may suggest a transient effect of the diets which disappears after long-term use of differing dietary fat sources. The highest amount of conjugated dienes was produced on the CONT diet. Negligible amounts of conjugated dienes were produced on

the CAN and LLNA diets although, there was appreciable variation among subjects in these groups. Increased conjugated diene formation also occurred in the SNFLR group but did not differ from the CAN and LLNA groups which suggests that diet had a marginal effect on LDL lipid oxidation in the NIDDM subjects as measured by total conjugated dienes produced. This finding is consistent with the absence of any effect of diet on LDL fatty acid composition for this period.

VI LDL Fatty Acid Composition

Because LDL-C levels are usually normal in well controlled NIDDM (Dimitriadis et al, 1995; Ronnemaa et al, 1995), abnormalities in LDL fatty acid composition are of interest as oxidized LDL is the species of LDL implicated in foam cell and plaque formation. The susceptibility of LDL to oxidation has been related to its LDL fatty acid composition. An aim of the present study was to examine the fatty acid composition of the LDL fraction of NIDDM subjects and to assess possible relationships between the composition of the LDL and its *in vitro* oxidative susceptibility. Research has shown that it may be feasible to favorably alter LDL fatty acid composition through dietary fat manipulation. This could prove beneficial to the NIDDM individual due to an increased susceptibility of LDL with high levels of PUFA to oxidative modification and, in turn, an increased risk of CHD. Three diet x gender interactions were observed for LDL fatty acids. C18:0 levels for women, increased slightly on the CAN, LLNA, and SNFLR diets during the 0-56 day period, whereas the levels on the CONT diet decreased. For men, C18:0 levels increased on the LLNA, SNFLR, and CONT diets but decreased on the

CAN diet. Whether these changes are physiologically significant is not known as C18:0 is a SFA which is considered to have neutral effects on lipoprotein levels.

C20:4 levels in the LDL fraction of women increased approximately the same amount on the CAN, LLNA, and SNFLR diets during the 0-56 day period compared to a decrease on the CONT diet. These results are consistent when compared to the PUFA intake of subjects. For men, C20:4 levels increased on the CAN and CONT diets while there was a decrease on the LLNA diet. There was a small decrease in C20:4 levels on the SNFLR diet but the change did not differ from those on the other diets. These results are inconsistent when compared to the PUFA intake of subjects, although the small number of males on the LLNA diet brings into question the validity of the comparisons among males, as well as the validity of the diet x gender interaction during this period.

C20:4 levels for women increased appreciably on the LLNA diet with smaller but statistically equivalent increases on the CAN and SNFLR diets over the 0-28 day period. These increases compared to a decrease on the CONT diet although the change on the CONT diet did not differ from those on the SNFLR and CAN diets. For men, the greatest increase was on the CONT diet. There was also an increase in C20:4 level on the CAN diet and a decrease on the LLNA diet. As a result, the changes on the CONT and CAN diets differed significantly from that on the LLNA diet. The change on the SNFLR diet did not differ from any of the other diets.

The same pattern for C20:4 was found during the 0-56 day period, suggesting that the change that occurred during the first month was sustained over the entire two month period. For women, the same pattern occurred for the CAN, SNFLR, and LLNA diets

over both the first month and the entire study period. However, the increase in C20:4 level on the CAN diet during the 0-56 day period resulted in a significant difference compared to the CONT group which was not apparent during the 0-28 day period.

Dimitriadis et al (1995) postulated that the reason NIDDM individuals have higher LA levels in the LDL cholesteryl ester fraction may be due to diet. However, in the current study, no evidence was found that CAN, LLNA, or SNFLR diets affected the amount of LA present in LDL. Within the context of the low-fat, high-CHO diet, the subjects in the present study did not receive differing amounts of LA. This coincides with the absence of change in LA concentration of the LDL fraction. Increased dietary PUFA have been observed to increase serum LA levels in NIDDM (Parfitt et al, 1993). Laitinen et al (1993) demonstrated that the serum fatty acid composition of recently diagnosed NIDDM subjects could be changed by diet therapy. LA enriched LDL obtained by dietary therapy produced an increase in LDL oxidation (Bonanome et al, 1992; Reaven et al, 1993). Increased LA has been suggested to result from impaired activity of insulin sensitive Δ-6 desaturase in NIDDM (Van Doomall et al, 1988). The appreciably low levels of LA and LNA consumed by the NIDDM subjects on the low-fat diet of the present study, as well as the negligible differences among the experimental spreads in these fatty acids, predicts that no significant differences would be observed. Differences in C20:4 levels may be the result of differences in animal protein intake over the study. The diet x gender interaction may be explained by the significant gender difference in protein intake during the study. However, the amount of protein consumed from animal sources during the study was not analyzed.

VII LDL Particle Size: Cor/Sur Volume Ratio, Radius, and Diameter

No significant diet effects were observed for any of the LDL particle size parameters. It is important to note that the LDL diameter incorporates both LDL core to surface volume ratio (cor/sur ratio) and radius values. Significant gender differences were observed for LDL cor/sur ratio, radius, and hence, diameter for the study period 0-56 days. This difference was not detected during the first or second months of the study which suggests that these changes may require a relatively long-term period for changes to occur. For men, there were larger increases in all three LDL particle size parameters than for women. LDL particle size has been found to be related to TAG concentrations. Small, dense LDL particles are usually accompanied by high serum TAG concentrations (Haffner et al, 1993; Lahdenpera et al, 1996; McNamara et al, 1987; Swinkels et al, 1987). These small, dense particles are thought to be more atherogenic than ordinary LDL (Austin et al, 1988a; Campos et al, 1992b; Crouse et al, 1985) most likely due to increased oxidative susceptibility (Tribble et al, 1992). Oxidative susceptibility of LDL has been shown to be highest in the most dense LDL subfractions (Chait et al, 1993; de Graff et al, 1993; Tribble et al, 1992) demonstrating an increased susceptibility to oxidative modification of small, dense LDL in NIDDM patients. These patients exhibit increased levels of small, dense LDL and an increased atherogenic lipoprotein profile (Dimitriadis et al, 1995). Oxidized LDL appears to be more atherogenic than native LDL particles (Steinberg et al, 1989).

There was a significant gender effect for TAG concentrations during the 0-28 day period (Table 7). TAG concentrations decreased for women, relating to the smaller

increase in LDL particle diameter observed for women. For men, TAG concentrations increased coinciding with an increase in LDL particle diameter. TAG concentrations have been found to be a major determinant of LDL particle size in NIDDM (Lahdenpera et al, 1996). However, for men, the findings of the present study do not coincide with the reported relationship between plasma TAG concentration and LDL particle size; the scientific literature suggests that smaller LDL particles are associated with higher TAG concentrations in NIDDM (Campos et al, 1992b; Feingold et al, 1992). In addition, the mean TAG concentrations for women decreased over the 0-28 day period and this decrease was associated with a smaller increase in LDL particle size than for men. This finding does not coincide with research suggesting that decreases in plasma TAG concentrations in NIDDM may lead to reversion of LDL particle size to the normal state (Barakat et al, 1990). More recently, it has been suggested that the effects of NIDDM and abnormalities in lipoprotein metabolism is the cause of changes in LDL particle size (Sing et al, 1995). Overall, there are no explanations for the contradictory findings of the present study.

The effect of NIDDM on LDL particle size is not fully understood. Whether NIDDM is an independent determinant of LDL particle size is controversial. Lahdenpera et al (1996) found LDL size was not independently associated with NIDDM but its influence was due to serum TAG concentration. Conversely, Haffner et al (1994) reported NIDDM to have an independent effect on LDL particle size in women but not in men. When LDL particles from men and women were analyzed separately by Singh et al (1995), the trends were the same for both genders, however, there was a significant effect

of diabetes only for female subjects which supports Haffner et al (1994) who reported that NIDDM had a greater effect on LDL particle size in women but not in men in their population based study. Lahdenpera et al (1996) found a significant association between LDL particle diameter and HDL cholesterol in NIDDM patients but not non-diabetic subjects. The mechanisms that modify LDL particle size in NIDDM individuals remains unclear. Several mechanisms may be involved in determining the lipoprotein profile of a NIDDM subject.

The exact importance of LDL particle size with regards to CHD risk is unknown. Men with CHD have been found to have significantly higher TAG concentrations and smaller LDL particles than men without CHD (Campos et al, 1992b). LDL particle size has been proposed to be a heritable trait which, however, is not fully expressed in premenopausal women and young men (Austin et al, 1988b). Generally, women are at lower risk for CHD than men. However, research has shown that female NIDDM patients are prone to greater incidence of CHD, suggesting an increased negative influence of NIDDM on lipoprotein patterns in women and loss of protection from estrogen hormones (Barrett-Connor and Wingard, 1983). The fact that some women in the study were pre-menopausal and others were post-menopausal may be an important consideration, although the effects of menopausal status on this parameter are not known. In the present study, there was large variability in the data for the LDL parameters. Different sizes and subfractions of LDL were not separated by the ultracentrifugation process carried out in the present study which may have lead to confounding of study results.

SUMMARY AND CONCLUSIONS

Non-insulin-dependent diabetes mellitus (NIDDM) is well known as a dysfunction of carbohydrate metabolism due primarily to insulin-resistance and hyperinsulinemia. Combined with a strong tendency towards obesity, these two factors result in abnormal lipid metabolism (elevated triglycerides and low HDL-C levels) and two to four times the risk of coronary heart disease and atherosclerosis. Since these factors are affected by dietary fat, the diabetic offers a unique model to investigate nutritional differences that may exist between regular canola oil and low LNA canola oil, in particular, any health benefits resulting from LNA. Low LNA canola oil has substantially greater oxidative stability compared to regular canola oil, providing a distinct marketing advantage to food manufacturers who are very interested in vegetable oils of high nutritional value and superior stability. If no nutritional benefit of LNA is established, plant breeders can accelerate efforts to reduce the LNA level of Canadian canola varieties.

The objectives of the study were to assess the effects of regular and low LNA canola oil on the blood lipid parameters of NIDDM subjects, with particular attention to any added benefits of LNA, and to determine if canola oil has nutritional benefits in the clinical treatment of NIDDM.

Based on the principles outlined above, a 56 day randomized block design study was conducted, using 16 male and 20 female free-living NIDDM subjects between the

ages 40 to 70 years. Subjects were recruited through the Health Sciences Centre Diabetes Education Centre (Winnipeg, MB), The Manitoba Diabetes Association Information Banner Newsletter, and television advertisements. Screening was conducted to ensure controlled blood pressure (<150/90 mm Hg), reasonable glycemic control, absence of cholesterol lowering medication, and intake of other high fat dietary products. Each participant continued to follow the current dietary recommendations for diabetes (a high-complex carbohydrate, low-fat diet; 30% total energy from fat) while assigned to one of four dietary fat groups: regular canola oil (CAN), low LNA canola oil (LLNA), sunflower oil (SNFLR) or their usual low-fat diet (control; CONT).

All added dietary fat sources were supplied to each subject in the form of a margarine spread and salad oil. Twelve-hour fasting blood samples were taken at 0, 28, and 56 days during the study. Blood parameters measured included glycosylated hemoglobin (GlyHB), plasma total and lipoprotein (LDL and HDL) cholesterol concentrations, as well as triglyceride concentration. Increased oxidative susceptibility of LDL has been shown to increase its atherogenicity, hence, LDL oxidative susceptibility was measured using copper-induced, *in vitro*, conjugated diene production and two indices of LDL oxidation: LDL oxidation rate and total amount of conjugated dienes produced. The fatty acid composition of the LDL fraction was quantified by gas-liquid chromatography to gain further insight into the relationship between LDL fatty acid composition and its oxidative susceptibility.

All dietary groups averaged slightly above 30% total energy as fat; the average percent of total energy from fat for all subjects was 33.8 $\pm$ 2.4% and the percent of total

added dietary fat from experimental spread/oil was 13.4 ± 7.8%. On a total energy basis, the average intake from experimental spread was 4.8%, 5.4%, 4.5 %, and 6.7% of energy for the CAN, LLNA, SNFLR and CONT diet groups, respectively. This represents very little energy obtained from the experimental spreads and is a limitation of the study.

Cholesterol intake was equal among all dietary fat groups. There was a high amount of variation within subject 3-day food records as well as between subjects for cholesterol intake.

No significant differences were found among dietary fat sources for plasma total, LDL and HDL cholesterol concentrations or plasma TAG concentration. Significant diet x gender interactions for LDL-C, HDL-C, and the total to HDL cholesterol ratio (TC:HDL-C ratio) were found at 0-56, 0-28, and 0-28 days, respectively. There were significant gender differences for TAG concentration during the 0-28 day period; TAG concentrations decreased for women and increased for men. For the TC:HDL-C ratio, significant differences among experimental diets were found during the 28-56 day period. The ratio decreased for the CONT group and statistical analysis indicated this change was significantly different from the slight decrease in the ratio for the CAN, LLNA, and SNFLR diets. There were negligible differences among dietary fat groups in LDL oxidation rate. A significant diet x gender interaction was observed during the study period 0-28 days and a significant diet effect was observed for the study period 28-56 days. LDL oxidation rate decreased equally on the CONT and LLNA diets, whereas there was an increase on the SNFLR diet and no change on the CAN diet. The second measure of LDL oxidative susceptibility was the total amount of conjugated dienes

produced. There were no differences among dietary groups in total diene production during the study periods 0-56 or 28-56 days. However, significant diet effects were observed for the study period 0-28 days. During this period, negligible amounts of conjugated dienes were produced on the CAN, LLNA, and SNFLR diets. The highest amount of conjugated dienes produced was on the CONT diet. The results for the assessment of LDL oxidative susceptibility suggests that the imposed diet changes on the study had marginal effects on LDL lipid oxidation in NIDDM subjects.

The LDL oxidative susceptibility findings are consistent with the absence of any effect of dietary groups on LDL fatty acid composition. No diet effects on LDL fatty acid composition were observed over the entire study. However, three diet x gender interactions were observed: C18:0 (0-56 days), C20:4 (0-56 days), and C20:4 (0-28 days). The significance of these findings is not known.

The findings of the present study demonstrated negligible differences among regular canola oil, low LNA canola oil, and sunflower oil with respect to their effects on the blood lipid parameters of NIDDM subjects, including TC, LDL-C, HDL-C, and triglyceride concentrations, as well as LDL oxidative susceptibility, fatty acid composition, and particle size. Thus, the null hypotheses of the study which stated there would be no nutritional benefits of these oils in the clinical treatment of NIDDM or their blood lipid parameters is confirmed. Furthermore, no unique nutritional advantages of LNA were established which confirms previous research suggesting there are no unique benefits of regular canola oil over low LNA canola oil, and supports the null hypothesis. The results of the present study, however, are limited by the difficulty experienced in

modifying the fat intake of NIDDM patients due to the limitations resulting from the restriction of a low-fat diet, namely 30% total energy as fat. The failure to appreciably alter the composition of the dietary fat could account for the absence of any significant changes in blood lipid parameters in response to the dietary fats in the present study.

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APPENDICES

Appendix 1. Subject Consent Form

HUMAN NUTRITION RESEARCH PROJECT

Department of Foods and Nutrition
University of Manitoba

CONSENT FORM

As a volunteer in the research project designed to study the nutritional properties of dietary fat, in particular canola oil, on blood total cholesterol and lipoprotein (e.g., LDL) cholesterol levels and factors involved in coronary heart disease, I am aware of the nature of the problem being investigated. I also acknowledge that I have been briefed in the project protocol and I am aware of the requirements to be fulfilled by the subjects, in particular conforming to the diet to which I will be assigned. I am aware that I will be assigned at random to one of four diets.

I am aware that blood samples will be collected at the beginning of the study, at 4 weeks and 8 weeks (the end of the study). I understand that approximately 30 mL of venous blood will be taken at each collection.

I understand that I will continue under the care of my physician throughout the study and that the investigators have contacted him or her concerning my participation in the study.

Dated the _____ day of _____ 19 ___ .

Signature: _____

Witness: _____

Appendix 2. Experimental Design: Subject Assignment and Recruitment Groups

	Gender and Dietary Fat Sources							
	Females				Males			
Age Group	CAN	LLNA	SNFLR	CONT	CAN	LLNA	SNFLR	CONT
40-45	32	35	5	21	33	4	13	-
46-50	6	24	31	25	11	17	23	7
51-55	28	19	27	15	2	9 [1]	29 [2]	8
56-60	22	30	20	36	18	10 [2]	39	16
61-70	12	1	14	37	26	34	3	38

[1] Subject number 9 dropped out of the study at week 4; data obtained from this subject was used when appropriate.

[2] Subject numbers 10 and 29 dropped out of the study after the first blood sampling day, therefore, no values exist for these individuals.

Recruitment Groups	Subject Numbers
I	1-8
II	9-16
III	17-23
IV	24-25
V	26-27
VI	28-34
VII	35-38
VIII	39

Appendix 3. Fatty Acid Composition of Experimental Spreads [1]

Fatty Acid [2]	CAN	LLNA	SNFLR
C10:0	1.21	1.22	1.13
C12:0	1.50	1.51	1.44
C14:0	4.85	4.90	4.60
C14:1	0.54	0.55	0.50
C16:0	15.05	15.24	15.81
C16:1	0.88	0.87	0.70
C18:0	5.01	5.31	6.84
C18:1	43.86	43.90	18.16
C18:2	14.77	19.15	47.06
C18:3	7.67	2.15	0.40
C20:0	0.29	0.27	0.16
C20:1	0.99	0.66	0.05
C22:0	0.07	0.05	0.12
C22:1	0.03	0.01	0.00

[1] Fatty acid amounts are expressed as a percentage of total fat.

[2] Carbon chain length : number of double bonds.

Appendix 4. Fatty Acid Composition of Experimental Oils [1,3]

Fatty Acid [2]	CAN	LLNA	SNFLR
C14:0	0.06	0.07	0.06
C16:0	4.36	4.34	6.01
C16:1	0.27	0.28	0.06
C18:0	1.38	1.75	4.28
C18:1	56.54	58.03	15.07
C18:2	23.00	30.39	73.56
C18:3	12.05	3.08	0.38
C20:0	0.38	0.26	0.18
C20:1	1.24	1.27	0.12
C22:0	0.12	0.09	0.14
C22:1	0.12	0.03	0.21

[1] Fatty acid amounts are expressed as percentage of total fat.

[2] Carbon chain length : number of double bonds.

[3] Canola oil, low LNA canola oil, and sunflower oil were donated courtesy of Can Amera (Winnipeg, MB).

Appendix 5. Fill-In Fat Intake Chart

Appendix 6. Diet Analysis: GC Settings

Gas Chromatograph:	Hewlett Packard 5890
Integrator:	Hewlett Packard 3392A
Column:	DB-225 capillary column 30 m x 0.25 mm, film thickness 0.25 microns, J & W Scientific (from Chromatographic Specialties)

GC Conditions:

- injector and detector temperatures 250°C
- oven temperature 205°C, isothermal
- column head pressure 18 psi
- He flow 50 ml/min (Diet analysis); 30 ml/min (platelet lipid analysis) (15 - 22 ml/min. overnight)
- gas gauge settings: Air 50 psi
  Hydrogen 60 psi
  Nitrogen 60 psi
  Helium 30 or 50 psi
- other GC settings: Range 2
  Zero OFF
  Attenuation 0
  Purge B ON
  Oven Max 230°C

Appendix 7. Health Sciences Centre Subject Blood Requisition Form

RESEARCH PROJECTS LABORATORY TEST REQUISITION

STUDY INFORMATION	PATIENT INFORMATION
Project name: "Assessment of the Nutritional Properties of Regular and Low Linolenic Canola Oil in Diabetes Mellitus" Researcher: Dr. Bruce M. McDonald Location: M514 Duff Roblin Bldg. Foods & Nutrition, U of M Telephone #: 474-8076 HSC Account No.: UofM P0#GO-95234-Z-J74 Project No.: 94.23 Institution Code: MBXXL1350	HSC #: Name: DOB: Loc: Dr.: Date: Time:

TESTS REQUIRED	SPECIMENS REQUIRED	TEST PROFILE COMPONENTS
Pre-coded Tests:		
LPP	1 Red Top Tube	Cholesterol, HDL Cholesterol, Triglyceride, Calculated LDL Cholesterol
~~APA~~	~~1 Red Top Tube~~	~~Apolipoprotein A1~~
~~APB~~	~~1 Red Top Tube~~	~~Apolipoprotein B~~
~~APLa~~	~~1 Red Top Tube~~	~~Apolipoprotein a~~
HBA1	1 Lavender Top Tube	Glycated Hemoglobin
Additional Tests:		

LAB ACCESSIONING INSTRUCTIONS:
1. Accession tests and/or profiles as listed above.
2. If Hematology tests are ordered, please place copy of test requisition in the black research tray.
3. Photocopy as required.

Appendix 8. LDL Phospholipid Content Calculations

(Bartlett, 1959; Folch, Lees, and Stanley, 1957; Van Heek and Zilversmit, 1991)

(1) LDL-PL (mM) =

$$\frac{[(Abs1 - Blk_y) \times (2/Std_x - Blk_y)] + [(Abs2 - Blk_y) \times (2/Std_x - Blk_y)]}{2} \times (2)(1)(0.3)(100)(25)/1000$$

Where:

Abs1 (2) = absorbance reading at 830 nm.

Blk_x = average of two blank absorbance readings.

Std_x = average of two standard absorbance readings.

Conversion from mM to mg PL/dl plasma:

LDL-PL (mg/dl) = LDL-PL (mM) x 80

Appendix 9. LDL Particle Size Calculations (VanHeek and Zilversmit, 1991)

(1) LDL Cor/Sur Volume Ratio [1] =

$$\frac{[\{\text{LDL-TAG}\} \times 1.093] + [\{\text{LDL-CE} \times 1.044\}]}{[\{\text{LDL-FC} \times 0.968\} + \{\text{LDL-PL} \times 0.97\} + \{\text{LDL-PRO} \times 0.705\}]}$$

(2) LDL Radius, r = (LDL Cor/Sur Ratio) (3) (21.5)

(3) LDL Diameter, dia = (LDL radius x 2) + (2 x 21.5)

[1] Abbreviations for the above calculations are as follows: LDL-TAG, LDL triglyceride content; LDL-CE, LDL cholesterol ester content; LDL-FC, LDL free cholesterol content; LDL-PL, LDL phospholipid content; LDL-PRO, LDL protein content. All values for the above mentioned parameters are expressed as mg/dl. Equations for the calculation of these parameters follow on the next page.

Appendix 9 (Cont'd): Equations for Calculation of LDL Triacylglyceride (TAG), Cholesterol Ester (CE), Free Cholesterol (FC), Phospholipid (PL), and Protein (PRO)

(1) LDL-FC, LDL-TC, and LDL-TAG (mM) =

$$\frac{[(Abs1 - Blk_x)(W/\{Std_x - Blk_x\}/X) + (Abs2 - Blk_x)(Y/\{Std_x - Blk_x\}/Z]}{2} \times LDL_f/LDL_i/0.9/1.5$$

Where:

LDL_f = final volume of LDL solution when diluted to 250µg LDL protein / mL.

LDL_i = initial volume of LDL solution before diluted to 250 µg LDL protein / mL.

Abs1 (2) = absorbance reading at 500 nm for both LDL total and free cholesterol, and 515 nm for LDL triacylglyceride.

Blk_x = average of two blank absorbance readings.

Std_x = average of two standard absorbance readings.

AND,

W = 5.15, 5.15, and 2 for LDL-FC, LDL-TC and LDL-TAG, respectively.

X = 5, 2.5, and 5 for LDL-FC, LDL-TC and LDL-TAG, respectively.

Y = 5.15, 5.15, and 2 for LDL-FC, LDL-TC and LDL-TAG, respectively.

Z = 5, 2.5, and 5 for LDL-FC, LDL-TC and LDL-TAG, respectively.

Appendix 9. (Cont'd)

(2) Conversion from mM to mg/dl:

LDL-FC (mg/dl) = LDL-FC (mM) x 38.6

LDL-TC (mg/dl) = LDL-TC (mM) x 38.6

LDL-TAG (mg/dl) = LDL-TAG (mM) x 85.6

(3) LDL-CE (mg/dl) = [LDL-TC (mg/dl) - LDL-FC (mg/dl)] x 1.7

(4) LDL-PRO (mg/dl) = [LDL-PRO (μg / mL) / 1000] x [100/0.9/1.5]

Appendix 10. F-values for Experimental Diet Effects on Mean Change in Glycosylated Hemoglobin Levels [1]

Experimental Diet	Sample Size (N)	R-squared	F-value	Prob
Study Period				
0-56 days	36	0.55 (0.16)	0.12 (4.91)	0.95 (0.03) [2]
0-28 days	36	0.60	0.03	0.99
28-56 days	35	0.52	0.17	0.91

[1] F-values and associated probabilities for Table 2.

[2] Unbracketed values represent the effects of diet on mean change in GlyHb. Values in brackets are R-squared, F and P- values for gender differences.

Appendix 11. F-values for Diet Effects on Mean Change in Plasma Total and Lipoprotein Cholesterol Concentrations [1]

Lipid Parameter	Sample Size (N)	R-squared	F-value	Prob
TC				
0-56	35	0.68	0.27	0.85
0-28	36	0.49	0.07	0.98
28-56	34	0.57	0.11	0.95
LDL-C				
0-56	31	0.96	9.51	0.05 [2]
0-28	33	0.89	1.69	0.28
28-56	32	0.86	0.99	0.48
HDL-C				
0-56	36	0.90	1.01	0.44
0-28	37	0.83	4.55	0.03 [2]
28-56	36	0.84	1.04	0.43
TAG				
0-56	32	0.95	4.37	0.09
0-28	35	0.13	4.20	0.05 [3]
28-56	33	0.87	3.96	0.09
TC:HDL-C				
0-56	34	0.81	0.46	0.72
0-28	36	0.76	4.12	0.05 [2]
28-56	33	0.98	6.28	0.04 [4]

[1] F-values and associated probabilities for tables 6, 7, and 8.

[2] Significant diet x gender interaction.

[3] Significant gender differences.

[4] Significant diet effects.

Appendix 12a. Linear Contrast for the Diet x Gender Interactions of LDL-C (0-56 Days)

LDL-C (0-56 days) Linear Contrast	Gender	T-statistic	Prob
CAN vs CONT	F	3.71	0.03
LLNA vs CONT	F	2.11	0.13
SNFLR vs CONT	F	2.84	0.07
CAN vs CONT	M	-3.68	0.04
LLNA vs CONT	M	-3.55	0.04
SNFLR vs CONT	M	-4.32	0.02

Appendix 12b. Linear Contrast for the Diet x Gender Interactions of HDL-C (0-28 Days)

HDL-C (0-28 days) Linear Contrast	Gender	T-statistic	Prob
CAN vs CONT	F	-1.31	0.22
LLNA vs CONT	F	1.94	0.08
SNFLR vs CONT	F	-1.23	0.25
CAN vs SNFLR	F	-0.08	0.94
LLNA vs SNFLR	F	3.18	0.01
CAN vs LLNA	F	-3.26	0.01
CAN vs CONT	M	-0.60	0.56
LLNA vs CONT	M	-0.21	0.84
SNFLR vs CONT	M	0.67	0.52
CAN vs SNFLR	M	-0.11	0.92
LLNA vs SNFLR	M	-0.89	0.40
CAN vs LLNA	M	0.83	0.43

Appendix 12c. Linear Contrast for the Diet x Gender Interactions of TC:HDL-C Ratio (0-28 Days)

TC:HDL-C (0-28 days) Linear Contrast	Gender	T-statistic	Prob
CAN vs CONT	F	1.85	0.10
LLNA vs CONT	F	0.13	0.90
SNFLR vs CONT	F	1.35	0.22
CAN vs SNFLR	F	-0.10	0.92
LLNA vs SNFLR	F	-1.22	0.26
CAN vs LLNA	F	1.72	0.12
CAN vs CONT	M	-1.74	0.12
LLNA vs CONT	M	0.38	0.71
SNFLR vs CONT	M	-1.48	0.18
CAN vs SNFLR	M	-0.26	0.80
LLNA vs SNFLR	M	1.87	0.10
CAN vs LLNA	M	-2.13	0.07

Appendix 12d. Linear Contrast for the Diet x Gender Interactions of LDL C18:0 Fatty Acid Composition (0-56 Days)

C18:0 (0-56 days) Linear Contrast	Gender	T-statistic	Prob
CAN vs CONT	F	1.29	0.24
LLNA vs CONT	F	0.72	0.49
SNFLR vs CONT	F	2.35	0.05
CAN vs SNFLR	F	-1.06	0.33
LLNA vs SNFLR	F	-1.63	0.15
CAN vs LLNA	F	0.57	0.59
CAN vs CONT	M	-1.95	0.09
LLNA vs CONT	M	1.79	0.12
SNFLR vs CONT	M	0.29	0.78
CAN vs SNFLR	M	-2.26	0.06
LLNA vs SNFLR	M	1.56	0.16
CAN vs LLNA	M	-3.42	0.01

Appendix 12e. Linear Contrast for the Diet x Gender Interactions of LDL C20:4 Fatty Acid Composition (0-56 Days)

C20:4 (0-56 days) Linear Contrast	Gender	T-statistic	Prob
CAN vs CONT	F	2.65	0.03
LLNA vs CONT	F	2.46	0.04
SNFLR vs CONT	F	1.69	0.14
CAN vs SNFLR	F	0.96	0.37
LLNA vs SNFLR	F	0.77	0.47
CAN vs LLNA	F	0.19	0.85
CAN vs CONT	M	0.15	0.88
LLNA vs CONT	M	3.84	0.006
SNFLR vs CONT	M	-1.79	0.12
CAN vs SNFLR	M	2.03	0.08
LLNA vs SNFLR	M	-2.38	0.15
CAN vs LLNA	M	4.10	0.005

Appendix 12f. Linear Contrast for the Diet x Gender Interactions of LDL C20:4 Fatty Acid Composition (0-28 Days)

C20:4 (0-28 days) Linear Contrast	Gender	T-statistic	Prob
CAN vs CONT	F	1.63	0.14
LLNA vs CONT	F	3.13	0.01
SNFLR vs CONT	F	1.62	0.14
CAN vs SNFLR	F	0.01	0.99
LLNA vs SNFLR	F	1.51	0.17
CAN vs LLNA	F	-1.50	0.17
CAN vs CONT	M	-0.24	0.81
LLNA vs CONT	M	-2.55	0.03
SNFLR vs CONT	M	-1.69	0.13
CAN vs SNFLR	M	1.54	0.16
LLNA vs SNFLR	M	-0.86	0.41
CAN vs LLNA	M	2.45	0.04

Appendix 12g. Linear Contrast for the Diet x Gender Interactions of LDL Oxidation Rate (0-28 Days)

LDL Oxidation Rate (0-28 days) Linear Contrast	Gender	T-statistic	Prob
CAN vs CONT	F	-1.98	0.11
LLNA vs CONT	F	-1.43	0.21
SNFLR vs CONT	F	-4.38	0.007
CAN vs SNFLR	F	2.54	0.05
LLNA vs SNFLR	F	3.27	0.02
CAN vs LLNA	F	-0.66	0.54
CAN vs CONT	M	-8.49	0.0004
LLNA vs CONT	M	-6.10	0.002
SNFLR vs CONT	M	-6.69	0.001
CAN vs SNFLR	M	-1.44	0.21
LLNA vs SNFLR	M	-0.37	0.73
CAN vs LLNA	M	-0.83	0.45

Appendix 13. Duncan and Tukey's Multiple Comparison Tests for Experimental Diet Effects : TC:HDL-C ratio (28-56 Days)

Experimental Diet	CONT	SNFLR	LLNA	CAN
Diet Group Means	-0.64	-0.21	-0.16	-0.08
Duncan's [1] Grouping	A	B	B	B
Tukey's [1] Grouping	A	AB	B	B

[1] Common letters in rows denote no significant differences ($p>0.05$) among experimental diets by DMCT and TMCT, respectively.

Appendix 14. F-values for Diet Effects on the Indexes of LDL Oxidation Rate and Total Change in Absorbance (Delta Absorbance)

LDL Oxidation Parameter	Sample Size (N)	R-squared	F-value	Prob
Rate				
0-56	35	0.92	3.56	0.08
0-28 [1]	33	0.99	20.71	0.003
28-56 [2]	33	0.96	6.13	0.04
Delta Abs				
0-56	36	0.84	0.90	0.48
0-28 [2]	34	0.23	2.85	0.055
28-56	33	0.93	2.42	0.18

[1] Significant ($p<0.003$) diet x gender difference.

[2] Significant ($p<0.04$ and $p<0.055$) diet effects for LDL oxidation rate (28-56 days) and total change in absorbance (0-28 days).

Appendix 15. Duncan and Tukey's Multiple Comparison Tests for Experimental Diet Effects : LDL Oxidation Rate (28-56 Days)

		Diet	Group	
	SNFLR	CAN	LLNA	CONT
Mean Change (Rate; ΔAbs/min) [1]	104	-18	-62	-70
Duncan's Grouping [2]	A	AB	B	B
Tukey's Grouping [2,3]	A	A	A	A

[1] Values are mean x 10^{-5} at Day 28.

[2] Means within the same row with the same letter are not significantly different ($p>0.05$).

[3] TMCT, generally has a higher type II (β) error rate.

Appendix 16. Duncan and Tukey's Multiple Comparison Tests for Experimental Diet Effects: Total Change in Absorbance (Delta Absorbance; 0-28 Days)

		Diet Group		
	LLNA	CAN	SNFLR	CONT
Mean Change (ΔAbs) [1]	-2	2	20	30
Duncan's [2] Grouping	A	A	AB	B
Tukey's [2,3] Grouping	A	A	A	A

[1] Values are mean x 10^{-3} at Day 0.

[2] Common letters in rows denote no significant differences among experimental diets ($p>0.05$) by DMCT and TMCT, respectively.

[3] TMCT generally has a higher type II (β) error rate.

Appendix 17. F-values for Diet Effects on Mean Change in LDL Fatty Acid Composition: 0-56 Days

Fatty Acid [1]	Sample Size (N)	R-squared	F-value	Prob
14:0	35	0.87	0.72	0.57
16:0	35	0.82	1.19	0.38
16:1	35	0.82	1.89	0.22
18:0 [2]	35	0.96	0.78 (5.44)	0.54 (0.03)
18:1	35	0.58	0.32	0.81
18:2	34	0.87	0.86	0.51
18:3	35	0.81	1.13	0.40
20:3	35	0.76	0.37	0.78
20:4 [2]	35	0.90	3.39 (5.40)	0.08 (0.03)
20:5	35	0.67	1.18	0.39
22:5	35	0.61	0.23	0.88
22:6	35	0.71	0.48	0.71

[1] Carbon chian length : number of double bonds.

[2] Unbracketed F-values and probabilities represent non-significant diet differences. Bracketed F-values and probabilities denote significant ($p<0.03$) diet x gender interactions.

Appendix 18. F-values for Diet Effects on Mean Change in LDL Fatty Acid Composition: 0-28 Days

Fatty Acid [1]	Sample Size (N)	R-squared	F-value	Prob
14:0	37	0.76	0.25	0.86
16:0	37	0.78	1.20	0.37
16:1	37	0.60	0.48	0.71
18:0	37	0.79	0.43	0.74
18:1	37	0.55	0.53	0.67
18:2	36	0.65	0.85	0.51
18:3	37	0.64	0.17	0.91
20:3	37	0.75	0.17	0.91
20:4 [2]	37	0.86	0.89 (6.87)	0.49 (0.01)
20:5	37	0.75	1.62	0.25
22:5	37	0.68	0.70	0.58
22:6	37	0.77	0.14	0.93

[1] Carbon chain length : number of double bonds.

[2] Unbracketed F-value and probability represent non-significant diet differences. The bracketed F-value and probability denote a significant ($p<0.01$) diet x gender interaction.

Appendix 19. F-values for Diet Effects on Mean Change in LDL Fatty Acid Composition: 28-56 Days

Fatty Acid [1]	Sample Size (N)	R-squared	F-value	Prob
14:0	35	0.75	0.52	0.68
16:0	35	0.73	1.02	0.44
16:1	35	0.78	0.32	0.81
18:0	35	0.75	0.55	0.67
18:1	35	0.82	0.15	0.93
18:2	35	0.84	0.97	0.46
18:3	35	0.70	0.85	0.51
20:3	35	0.70	0.32	0.81
20:4	35	0.63	0.16	0.92
20:5	35	0.68	0.14	0.93
22:5	35	0.80	0.14	0.93
22:6	35	0.39	0.08	0.97

[1] Carbon chain length : number of double bonds.

Appendix 20. F-vlaues for Diet Effects on LDL Particle Size Parameters: Cor/sur, Radius, and Diameter

Particle Size Parameter	Study Period	Sample Size (N)	R- squared	F-value	Prob
Cor/Sur [1]					
	0-56 [2]	36	0.15	4.71	0.04
	0-28	35	0.75	0.32	0.81
	28-56	34	0.76	0.68	0.59
Radius [1]					
	0-56 [2]	36	0.16	4.73	0.04
	0-28	35	0.75	0.33	0.80
	28-56	34	0.76	0.73	0.57
Diameter [1]					
	0-56 [2]	36	0.15	4.70	0.04
	0-28	35	0.75	0.35	0.79
	28-56	34	0.76	0.75	0.56

[1] Significant ($p<0.05$) gender differences for the study period 0-56 days.

[2] For the study period 0-56 days, R-squared, F-value, and probability values represent significant gender effects as no significant diet differences were found.