Oxidative Stress and Risk of Cardiovascular Disease Associated with Low- and High-Monounsaturated Fat Portfolio Diets

By

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Abstract

The objective was to assess the effect of a high-monounsaturated fat (MUFA) dietary portfolio of cholesterol-lowering foods on oxidative stress and cardiovascular risk. Twenty-four hyperlipidemic subjects followed a very low-saturated-fat therapeutic control diet for 4 weeks after which they were randomized to receive the dietary portfolio, consisting of soy protein (20g/1000kcal), viscous fibre (10.3g/1000kcal), plant sterols (2-3g) and almonds (21.5g/1000kcal), in combination with high- or low-MUFA (25.9% and 12.9% MUFA, respectively) for the next 4 weeks, where MUFA replaced 13.0% of dietary carbohydrate. On high-MUFA, there were significantly greater increases in HDL-C and apoA1 and significantly greater reductions in total:high-density lipoprotein cholesterol (total:HDL-C) ratio and high-sensitivity C-reactive protein (hs-CRP) compared to the low-MUFA dietary portfolio. In all diets there were significant increases in protein thiols and reductions in conjugated dienes and thiobarbituric acid reactive substances (TBARS) measured in the LDL-fraction, however no difference between the high- and low-MUFA diets.

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8-OHdG – 8-Hydroxydeoxyguanosine
ABC transporter – ATP-binding Cassette Transporter
AHA – American Heart Association
apoA1 – apolipoprotein A1
apoB – apolipoprotein B
BMI – Body Mass Index
CD – Conjugated Dienes
CE – Cholesterol Ester
CETP - Cholesterol-Ester Transfer Protein
CHD – Coronary Heart Disease
CV – Coefficient of Variance
CVD – Cardiovascular Disease
DASH – Dietary Approaches to Stop Hypertension
DNA – Deoxyribonucleic Acid
DTNB – 5,5’-Dithio-bis 2-Nitrobenzoic Acid
FDA – Food and Drug Administration
HDL – High-Density Lipoprotein
HDL-C – High-Density Lipoprotein Cholesterol
HL – Hepatic Lipase
hs-CRP – high-sensitivity-C-Reactive Protein
IVUS – Intravascular Ultrasound
IL-6 – Interleukin-6
LCAT – Lecithin Cholesterol Acyltransferase
LDL – Low-Density Lipoprotein
LDL-C – Low-Density Lipoprotein Cholesterol
LPL – Lipoprotein Lipase
LRC-CPP – Lipid Research Clinic Coronary Primary Prevention
MDA – Malondialdehyde
MET – Metabolic Equivalent of Tasks
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1. Introduction
1. Introduction

Cardiovascular disease (CVD) is the leading cause of death in Canada, accounting for approximately one-third of all deaths, and is the largest economic burden of disease (1). For prevention and treatment of CVD, attention to diet and lifestyle has been continuously growing as Canadians are being encouraged to follow what has been termed a “heart-healthy diet” (2, 3). Essentially this means limiting intake of saturated fat, choosing leaner meats, low-fat dairy, and increasing consumption of fruits, vegetables and whole grains as is recommended by many organizations, including the American Heart Association (AHA) and the Heart and Stroke Foundation of Canada (4, 5).

Over the past few decades, specific foods have been looked at for their potential roles in reducing the risk of CVD by targeting one or more of the associated risk factors. Elevated blood cholesterol is one such risk factor. It is estimated that about 40% of Canadians have high blood cholesterol levels (6). The National Institutes of Health in their Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel, ATP, III Final Report) encourages the use of therapeutic dietary options for enhanced reduction of low-density lipoprotein cholesterol (LDL-C), such as plant sterols and viscous (soluble) fibres (7). These dietary options have been previously combined with other foods, including almonds and soy protein, which have also demonstrated cholesterol reduction, into a dietary intervention termed the dietary portfolio, which has been shown to effectively reduce cholesterol levels to a similar extent as the therapeutic dose of first-generation statins (28.6% vs. 30.9%, respectively) compared to a low-fat control diet (8.0%) (8).

Another risk factor for CVD which has been given much attention is oxidative stress. Many studies have been conducted on the effects of varying dietary antioxidants on oxidative stress levels and risk of CVD with conflicting results, indicating the need for further investigation (9). However, it is possible that through the consumption of whole foods and the interactions between vitamins and nutrients that a greater benefit may be observed. Dietary portfolio
studies have also shown significant reductions in high-sensitivity C-reactive protein (hs-CRP) and increases in protein thiols, demonstrating the potential to improve oxidative and inflammatory status (10-12). In addition to antioxidant-rich foods, the lipid factor high-density lipoprotein cholesterol (HDL-C) has been associated with improvements in lipid oxidation, as well as anti-inflammatory and antithrombotic activity. High HDL-C levels have been associated with reduced oxidative stress and conversely, low HDL-C has been associated with increases in oxidation (13-15). Methods known to increase HDL-C levels include increased dietary fat intake, as well as exercise, smoking cessation and moderate alcohol intake (16, 17). As a result of recommended dietary restrictions on saturated fatty acid (SFA) and polyunsaturated fatty acid (PUFA) intake, monounsaturated fatty acid (MUFA) intake is recommended as the dietary method of increasing HDL-C, which can be achieved through increased consumption of foods rich in MUFA such as avocados, high-MUFA oils including olive and canola oil, as well as nuts and seeds.

In the present study, MUFA was added to an effective dietary portfolio of cholesterol-lowering foods to determine if further reductions could be made in CVD risk, particularly by targeting the oxidative and lipid risk factors.
2. Literature Review
2.1 CVD Risk Factors

2.1.1 Hyperlipidemia

Cardiovascular disease (CVD) is a leading cause of death in North America. Although rates of death due to heart disease have declined by 25% in the past decade, it still accounts for approximately one third of all deaths in both Canada and America and is expected to rise due to increasing rates of obesity and diabetes, which are known risk factors for CVD (6, 18). Other risk factors include hypertension, smoking, oxidative stress and inflammation, and high blood cholesterol, especially elevated LDL-C. Due to the prevalence of CVD, organizations exist to continuously evaluate scientific evidence in order to present clinical guidelines on how best to prevent and treat risk factors. The National Cholesterol Education Program Expert Panel and the Canadian Cardiovascular Society have developed treatment recommendations, which are updated regularly with evidence from emerging trials, in order to control hyperlipidemia and reduce the risk of heart disease. Their recommendations are based on risk of developing coronary heart disease (CHD), which is calculated, for example, from an equation developed as a result of a large epidemiological study, the Framingham Heart Study. The Framingham predictive risk equation is used to predict 10-year risk of CHD and includes the risk factors age, total cholesterol (total-C), HDL-C, systolic blood pressure, and cigarette smoking (19). LDL-C remains the primary lipid target because many studies over the past 3 decades have demonstrated that through the use of statins, LDL-C levels could be reduced with a corresponding reduction in risk of CVD (20, 21). One meta-analysis found that for every 1.0mmol/L decrease in LDL-C, there is a corresponding reduction of 21% and 12% in major cardiovascular disease events and all-cause mortality, respectively (20). However, not only the quantity, but the quality of the LDL-C plays a very important role in the risk of CVD since when coupled with an increased oxidative and inflammatory environment, can result in the development of atherosclerosis, which is one of the most prevalent forms of CVD (22). Research focused on reducing oxidative stress has been inconsistent, unlike the majority of research on the other risk factors. For example, blood pressure reduction has been demonstrated in the successful DASH (Dietary Approaches to Stop Hypertension) trial and can also be controlled through...
pharmaceuticals (23). Also, there are many methods available for smoking cessation and exercise can reduce obesity, which coupled with avoiding excess carbohydrate, especially simple sugars or choosing foods with low glycemic index (GI), can help in the management or prevention of diabetes (24, 25). Research, however, on reducing oxidative stress, including the trials on antioxidant supplementation, is still very controversial, therefore requires further investigation.

**2.1.2 Oxidative Stress**

Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS), which occurs naturally from oxidative metabolism, and antioxidant defences (26). ROS are unstable molecules, such as superoxide, hydroxyl and hydroperoxyl radicals, with an unpaired electron which can readily extract electrons from other molecules, thus behaving as oxidants (27). They can act beneficially by killing pathogens through phagocytes in the immune system, but can also elicit cytotoxic effects (28). When the amount of ROS rises above normal levels, which has been associated with many chronic disease states including hypercholesterolemia and atherosclerosis, the antioxidant system of the body is overwhelmed, resulting in the oxidation of particles such as proteins and lipids (29). The oxidation of the lipid particle LDL-C is known to increase its atherogenicity since oxidized LDL (ox-LDL) is recognized by macrophages and taken up via scavenger receptors. The macrophages are then thought to become foam cells which form plaque in the arteries and thus, the development of atherosclerosis (22, 30, 31) (Figure 1). Therefore, the oxidative modification of LDL-associated lipids is directly involved in the initiation process of atherosclerosis (32).
2.1.2.1 Measures of Oxidative Stress

Due to the very short half-lives of ROS, damage is measured via causally associated molecules such as those resulting from protein and lipid oxidation (34-37) (Figure 2).
Figure 2. Oxidative modification of cellular macromolecules (36).

Protein oxidation is measured by the oxidation of side chains. The DTNB [5', 5'-dithio-bis(2-nitrobenzoic acid)] assay is a common method used to measure the
oxidation of sulphur containing amino acid side chains (cysteine and methionine) because the DTNB molecule reacts with the thiol groups (-SH) (Figure 3). When there is increased protein oxidation, less thiol groups will be detected since they will be already oxidized into disulfide bonds (S-S).

![Chemical reaction](image)

Figure 3: Reaction of a thiol group from a sulphur-containing amino acid with DTNB as a measurement of the amount of unoxidized proteins (38).

For lipids, the oxidation of LDL is of great interest because of its association with the development of atherosclerosis. There are multiple ways of measuring oxidized LDL, two of which include via conjugated dienes (CD) and thiobarbituric acid reactive substances (TBARS) in LDL extracts precipitated from serum. In the former, the amount of CD is measured directly by spectrophotometric determination of CDs in the extracted LDL lipids (39). In the latter, the lipid peroxidation product malondialdehyde (MDA) is measured through its reaction with thiobarbituric acid, which is added to the serum, and also read spectrophotometrically (40). Considering the association between ox-LDL and disease states including hypercholesterolemia, diabetes and the development of cardiovascular disease (41, 42), many studies have investigated the role of antioxidants in minimizing oxidative damage and reducing the risk of CVD.

2.1.2.2 Supplemental Use of Antioxidants

Studies investigating the supplemental use of antioxidants have demonstrated conflicting results. Some studies have demonstrated reductions in oxidative damage resulting from antioxidant use, such as a reduction in ox-LDL with high doses of vitamin E supplementation (43-46). However, most large trials conducted with antioxidant supplementation have found no effect, such as the MRC/BHF (Medical Research Council and British Heart Foundation) Heart Protection Study which assessed long term supplementation of vitamin E, C and beta-carotene daily in subjects at high risk of CHD (47). Other studies have demonstrated harmful effects, such as the increased risk of
lung cancer and cardiovascular disease in smokers supplemented with β-carotene (48), or the HOPE (Heart Outcomes Prevention Evaluation) Study which found an increase in heart failure in high risk subjects given 400IU of vitamin E daily for a mean of 7 years (49). It must be questioned, however, whether these studies were targeting the oxidative stress risk factor in the appropriate way. It is known that antioxidants may become pro-oxidants in an oxidative environment, therefore, instead of giving single vitamin supplements to high risk subjects who are under high oxidative stress, it may be more effective to target the environment itself as a whole. This may be accomplished by consumption of foods rich in vitamins in their whole form as it may be through the interaction of all food components where protection seen in epidemiological studies may be attained. Some support for this idea includes the results of the DASH trial which tested a diet rich in fruit, vegetables, whole grains and low-fat dairy and not only demonstrated significant reductions in blood pressure, but in further analyses, found significant reductions in markers of oxidative damage (23, 50, 51). Additionally, the main sources of many vitamins are plant foods, as well as nuts, seeds, and vegetable oils for vitamin E, therefore, the benefits noted in epidemiological studies may be the result of multiple vitamins working together or in combination with other nutrients present, such as phytochemicals, fibre, or polyunsaturated and monounsaturated fats (52). Thus, further investigation into the consumption of whole foods rich in vitamins and nutrients is required to allow for possible synergistic activity and the potential promotion of a less oxidative environment and ultimately, reduction in CVD risk.

2.1.3 HDL-C

Risk factors other than LDL-C are increasingly receiving attention due to the residual risk for CVD that remains once LDL-C has been effectively lowered. This includes the growing area of research on low HDL-C and risk of CVD independent of LDL-C, total-C and TG. Many studies have found that for every 0.026mmol/L increase in HDL-C, there is a corresponding 1-3% reduction in CVD risk and that even when LDL is reduced or total-C levels are low, those with lower HDL-C are at a much greater risk of developing CVD (53-61). As a result of the inverse correlation between HDL-C levels and risk for CVD, the activities of HDL-C have been investigated. HDL-C is known for its
facilitating role in reverse cholesterol transport (RCT) whereby excess cholesterol from the peripheral tissues is transferred from the plasma to the liver where it is either recycled or excreted from the body through bile (62). Additionally, HDL-C has many effects on the endothelium and antithrombotic actions (63, 64). However, its ability to inhibit the oxidative modification of LDL via its potential antioxidative activity may be of greater interest since oxidized LDL plays a central role in the initiation and propagation of atherosclerosis (65-67). Mechanistically, there are many possible pathways by which HDL-C may inhibit LDL oxidation, such as through the HDL-associated proteins paraoxonase 1 (PON1) (14, 68) and platelet-activating factor acetylhydrolase (PAF-AH) (69). The former protects against LDL oxidation, reverses biological effects of oxidized LDL, inhibits the oxidation of HDL thereby preserving its function, and has been associated with reduced risk of CVD (13, 70-75). The later attenuates proinflammatory activity of PAF and functions as an antioxidant by hydrolyzing oxidized phospholipids which result from the oxidation of LDL (76-78).

Another important component of HDL-C is its associated apolipoprotein, apoA1. ApoA1 is a useful clinical measure because it can be measured directly with little error and is a reflection of the anti-atherogenic HDL-C. It plays a central role in RCT, as it is responsible for its initiation by picking up cholesterol from the periphery and delivering it to the liver in HDL particles, and it has also been shown to have anti inflammatory and antioxidant effects (79). Recently, apoA1, as well as the apolipoprotein associated with LDL-C, apoB, have been called more informative risk indicators than their lipoproteins or other lipids (79, 80). Two very large epidemiological studies, the AMORIS (Apolipoprotein Mortality RISk) and INTERHEART studies (A Global Study of Risk Factors for Acute Myocardial Infarction) found that there was a very strong direct relation between the apoB:A1 ratio and fatal or acute myocardial infarction and that it was the strongest lipid ratio predictor of CVD risk (81, 82). Interest in apoA1 began in the early 1980s when it was discovered that a small group of people in Italy carried a mutation of the apoA1 gene that resulted in low HDL-C and apoA1 and high TG but low rates of CVD, which they termed apoA1 Milano (83, 84). Studies began in the 1990s with the recombinant version of apoA1 and those in animals demonstrated regression of atheroma volume and promotion of more stable plaques (85-87). A few studies have
been conducted in humans also demonstrating significant regression of atherosclerosis when given recombinant apoA1\textsubscript{Milano} complexed with phospholipids to mimic properties of nascent HDL (85, 88). It appears that the mutation results in a form of apoA1 with elevated performance of the activities of normal apoA1. Additionally, a number of prospective cohort studies have concluded that HDL-C and apoA1 are strong predictors of CVD risk, with some suggesting apoA1 is the stronger predictor (58, 89-95). As a result, some countries have expanded their clinical guidelines for decreasing CVD risk to include apoA1, including Sweden and Norway (96). Therefore, apoA1 and HDL-C remain potential targets for anti-atherosclerotic therapeutic strategies.

In order to test whether elevation in HDL-C can improve CVD risk, methods of increasing HDL-C have been elucidated. Those that have demonstrated success include exercise, smoking cessation, weight loss, moderate alcohol intake and increased MUFA consumption (16, 17, 97-99) and they have also demonstrated the potential to reduce CVD risk (100-104). From a dietary approach, MUFA\textsubscript{s} could be easily incorporated into the diet as they are found naturally in oils, such as olive and canola, as well as in nuts, seeds and avocados.

2.2 Foods with cholesterol-lowering and anti-oxidative potential

2.2.1 Nuts

The many health benefits of nuts have fuelled investigations into their effects on CVD risk with many having an associated decreased risk (105-109). Some studies indicated that a CVD risk reduction of 35% or more may be obtained with increased nut consumption (≥ 2-5 servings per week) (110-112). As a result, nuts have been permitted a health claim for heart disease risk reduction by the FDA stating that as part of a diet low in saturated fat and cholesterol, consumption of 42g (1½ oz) of almonds per day may reduce the risk of heart disease (113). In addition to studies looking at the overall effect of nut consumption on CVD risk, many studies have investigated potential ways by which nuts target risk factors, including effects on lipids.

LDL-C has been shown to be significantly reduced by the consumption of nuts, primarily almonds and walnuts (114, 115). Consumption of between 68 to 100g per day
of almonds has been shown to result in LDL-C reductions of 7 to 29% (116, 117). Even consuming a handful (approximately 1 ounce) of almonds a day has demonstrated reductions in LDL-C around 4-5% (118). Almonds are an excellent source of the antioxidant vitamin E, phytochemicals, flavonoids, fibre, protein, and MUFA, however exactly which part of the nut results in the lipid benefit is unknown. Some studies comparing the effects of the whole nut to the oil from the nut have demonstrated similar reductions in total- and LDL-C, therefore there is the potential for the effect to arise from the type of fats found within the almond (115), most of which is MUFA. In addition to the lipid lowering effects, almonds have associated with a reduction in oxidative damage.

The antioxidant activity of almonds has been demonstrated in reductions in LDL oxidizability, inflammatory molecules and endothelial dysfunction (119). Acute studies have demonstrated that whole almonds decreased the susceptibility of lipids to oxidation and increased the total antioxidant capacity, and did so more than compared to walnuts (120). Additionally, studies investigating the effects of the skins of almonds have demonstrated that the variety of polyphenols within have anti-oxidant activity against specific radicals (121, 122). Due to the fact that the different components of almonds have potential antioxidant activity, greater oxidative reduction may result from the consumption of whole almonds thereby targeting a larger variety of radical types and the health conditions that may arise from each.

### 2.2.2 Soy Protein

The effect of soy protein on blood cholesterol has been of interest since the early 1900s when the idea emerged that animal protein had negative consequences in the arteries and thus a search for a plant protein replacement began (123). Compared to lean meat protein, some studies have demonstrated soy protein significantly reduced LDL-C around 10% versus 5%, suggesting a specific effect of proteins on cholesterol transport and metabolism (123, 124), whereas other studies have reported significant reductions of only 5-6% (125-131). Studies comparing the effects of soy protein to casein protein express similar results where soy protein effective reduced LDL-C 16% more than did the casein protein treatment (132). A meta-analysis conducted in 1995 indicated a 12.5% reduction in LDL-C as a result of an average 47g per day of soy
protein whereas subsequent studies did not support as great a reduction (126, 133-
135). However, in an article by Sirtori in 2007, who conducted many of the early studies
expressing the beneficial effects of soy protein, he re-evaluated the more recent studies
using a ‘nomogram’ prepared on the basis of initial cholesterol concentrations in the
1995 meta-analysis and concluded that the studies done in the past 10 years are in
agreement with the conclusion of that meta-analysis (136). Additionally, a recent meta-
analysis by Harland concluded that modest soy protein intakes of around 25g per day,
almost half as much as the 1995 meta-analysis, resulted in highly significant reductions
in LDL-C of 6%, which supports the previous meta-analysis (131).

There has been much debate over the effectiveness of soy protein versus soy
isoflavones, and over the effectiveness of highly processed soy products. However, soy
isoflavones have not acquired as much support for their cholesterol-lowering effects and
there are many studies indicating the greater the processing of soy products, the less
effective in cholesterol reduction (133-135, 137, 138). Therefore, the most effective
method of cholesterol-reduction with soy foods is by using whole soy foods which are
least processed.

Soy protein also has potential for reducing lipid oxidation. Many studies done in
animals (139-144) and in humans (145, 146) have reported significant antioxidant
activity resulting from soy protein consumption, possibly by up-regulating LDL-receptor
machinery in the liver, resulting in decreased LDL levels and LDL oxidation. Studies
have demonstrated about a 9-16% reduction in conjugated dienes in the LDL fraction
with intakes of soy foods of around 33g/d (147, 148) Again, there is debate whether it is
the soy isoflavones that contribute the antioxidant properties, however in this respect,
there is more evidence supporting its role (149, 150) in addition to those on whole soy
foods (147, 148). Overall, epidemiological studies suggest the soy consumption is
associated with a reduced risk of CVD (151).

2.2.3 Viscous Fibre
Dietary fibres can be classified based on their solubility. The insoluble fibres include
lignins and cellulose, whereas soluble fibres, also known as viscous fibres, include
natural gel-forming fibres such as pectins, gums and mucilages. Overall, dietary fibre is
associated with reduced risks for many diseases including many cardiovascular
diseases (152, 153). However, much of the attention on fibres is on the soluble fibres
including oats, pectins, guars and psyllium and their LDL-C lowering abilities. Common
foods high in viscous fibre include whole oats, barley, psyllium powder, oat bran,
eggplant, and okra. Many studies have demonstrated reductions of 7-9% in LDL-C and
that intakes of only 3g of soluble fibre can have significant reductions in LDL-C of
0.13mmol/L (154) and the consumption of fibre from 3 apples or 3 bowls (28g) of
oatmeal can result in total-C reductions of 2% (154, 155). There are a few possible
mechanisms of effect including increased bile acid output resulting in less reabsorption
of cholesterol and greater usage of cholesterol for hepatic bile acid synthesis (156,
157), inhibition of hepatic fatty acid synthesis by products of fermentation (158), and
changes in intestinal motility (159). Studies of soluble fibre have also shown reductions
in small LDL subfractions which are associated with more atherogenic conditions (160).

In addition to the cholesterol-lowering effect of viscous fibres, other
cardiovascular benefits include reductions in the inflammatory marker CRP (161, 162)
and blood pressure (163, 164) and some studies have demonstrated the potential for
antioxidant activity. Animal studies have demonstrated guar gum can increase
antioxidant protein expression and therefore decrease oxidative stress-induced arterial
injury (165). Also, since oxidative stress can result from inflammation, the anti-
inflammatory potential of fibre is also of interest. In the Iowa Women’s Health Study,
whole grain consumption was associated with a reduced risk of death attributed to
inflammatory diseases which were noncardiovascular of >35% for those who reported
the highest intake (≥ 22.5 servings per week) and since whole grains are an excellent
source of fibre, there is potential for fibre to play a role in reducing inflammation (166).
The same group analyzed the total antioxidant capacity in over 1000 food samples
obtained from the US Department of Agricultural National Food and Nutrient Analysis
Program and found that several whole grain products were at the top of the ranked list
(166). This may be the result of increased enterlactone concentrations associated with
whole grain intake, as a product of lignin precursors within whole grains, which have
anti-oxidative activity (166).
Overall, the multifactorial effects of viscous fibre and its large range availability make it an attractive addition to obtain greater cardiovascular health.

2.2.4 Plant Sterols and Stanols

Plant sterols and stanols are structurally similar to cholesterol with sterols being found naturally in plant foods such as vegetable oils, nuts and seeds, and stanols being commercially available hydrogenated sterols (167). They are believed to exert their cholesterol-lowering abilities by decreasing the absorption of dietary and biliary cholesterol, thus increasing cholesterol synthesis and the activity of LDL-receptors and ultimately reducing serum LDL-C concentrations (167, 168). Studies have demonstrated these effects over the past 40 years with plant sterols/stanols enriched in vehicles such as margarines, dairy products and orange juice and have shown that for consumption of 3.4g of esterified plant sterol/stanol (or 2.1g plant sterol/stanol) reductions in LDL-C range from 5 to 16% (169-172), with margarines, the more common vehicle, averaging about 11% reduction (169). As a result, the United States Food and Drug Administration (FDA) granted plant sterols health claim status for CHD risk reduction (113) and NCEP ATP III recommends them for cholesterol reduction (19). Although there have been some studies questioning the safety of plant sterols (173-177), many studies have alleviated those concerns (178-182) and a recent review assessing their efficacy and safety concluded that they are important for cholesterol-lowering and good heart health (183).

There is also some evidence for potential anti-inflammatory and antioxidative effects of plant sterols. Some studies have shown they may inhibit the secretion of inflammatory mediators, such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), although most of these studies have been done in animals (184). The studies on their antioxidative effects have been done in humans and demonstrate a reduction in ox-LDL with 2-3g plant sterol consumption, thus suggesting the potential to protect LDL from oxidation (185, 186). However, some studies have shown no effect (187).

As a result of their cholesterol-lowering ability and the potential for anti-inflammatory and antioxidative activity, plant sterols may contribute to reducing CVD risk.
2.2.5 The Dietary Portfolio

In the late 1990s with the growing interest in diet in the management of elevated blood lipids and the many studies investigating the cholesterol-lowering effects of particular foods, many organizations began recognizing key foods which had demonstrated effectiveness in cholesterol reduction. The NCEP ATP was recommending 2g/d of plant sterols and 10-25g/d of viscous fibre (19) and the AHA was encouraging the consumption of soy proteins (188) for the management of elevated cholesterol. The FDA had also approved health claims for plant sterols (113), viscous fibres (189) and soy protein (190). Mechanistically, studies had shown that viscous fibres increased bile acid losses (191-193), plant sterols increased fecal cholesterol losses (194, 195) and soy proteins decreased hepatic cholesterol synthesis and increased LDL-receptor-mediated cholesterol uptake (196, 197). With this information, Dr. David Jenkins saw the potential for greater cholesterol reduction through dietary means and therefore combined these foods into what he termed the “dietary portfolio”. In a preliminary study combining 1.2g/1000kcal plant sterols, 8.3g/1000kcal viscous fibres, and 16.2g/1000kcal soy protein, as well as 2.9g/1000kcal of almonds as an added source of vegetable protein and because it also had previously demonstrated the potential for cholesterol reduction (114), Dr. Jenkins found this dietary portfolio had an additive effect in lowering LDL-C by 29% (198). With these results, and an FDA health claim for nuts which encouraged an increase in the contribution of almonds to 16.2g/1000kcal in the dietary portfolio, a series of studies assessing the many benefits of this dietary portfolio began (8). When tested alongside a first generation statin, the dietary portfolio demonstrated LDL-C reductions of a similar magnitude (30.9% and 28.6%, respectively), as well as reductions in hs-CRP (33.3% and 28.2%, respectively) (10). When assessed for long-term effects under real-world conditions, as opposed to metabolically controlled, 14% and 12% reductions in LDL-C were seen at 3 months and 1 year, respectively, with >30% of subjects maintaining a reduction >20% (199). Additionally, significant reductions in blood pressure (200), haematological indicies
(201) and reductions in the smallest and most atherogenic subclass of LDL-C (202) have also been demonstrated over the years. Most recently, strawberries were added to the dietary portfolio and demonstrated significantly improved palatability and reductions in TBARS as a measure of oxidative stress, while maintaining reductions in blood lipids, therefore suggesting that added berries may improve the overall utility of the diet (12). It is thus of interest to continue to explore methods of improving this effective cholesterol-lowering diet to further reduce the risk of CVD.

### 2.2.6 Monounsaturated Fatty Acids (MUFAs)

With interest in determining the best replacement for SFA, many studies have looked into the effect of MUFA compared to carbohydrates and PUFA. In terms of effects on blood lipids, all three of the above food components are effective in cholesterol reduction when compared to the average American diet (203, 204). However, when looking into the relative effects on markers of oxidation, many studies have demonstrated a significant difference with MUFA being the better choice.

MUFA consumption has been found to result in less oxidative modification of LDL-C compared to when PUFAs are consumed (205), and additionally, when carbohydrate is replaced by MUFA there are reductions in such measurements of oxidation as LDL oxidation lag phase and TBARS (203, 206, 207). There are also many studies which conclude that MUFAs are the preferred substitution due to a greater potential risk reduction for CVD (203, 208). The AHA also indicates in its statement that there are prospective observational studies which document that diets rich in MUFAs are associated with a decreased risk of CHD (100).

MUFAs are most commonly found in oils such as olive and canola, as well as in nuts, seeds and avocados. Many studies on olive oil on risk of CVD have demonstrated reductions in markers of oxidative stress. One study which fed participants olive oil at real-life doses (25mL/day), found a significant increase in the oleic/linoleic acid ratio in LDL-C and that with every 1 unit increase in the ratio, there was an associated decrease of 4.2ug/L in plasma isoprostanes, an in vivo measure of ox-LDL (209). Other studies have also demonstrated how an increase in the amount of oleate in the diet can reduce the susceptibility of LDL to oxidative modification (210).
There is also interest in MUFA because of the potential to raise HDL-C, which is a risk factor for CVD when levels are low. Although some studies have found no effect of increased MUFA consumption on HDL-C levels (211-214), there are many studies which provide support for their potential (203, 215, 216). In the early 1970s when the interest in HDL-C as a cardioprotective factor began and the search for a suitable replacement for SFA was of interest, large quantities of PUFA (as much as 20 to 30% of total calories) were demonstrated to result in great reductions in HDL-C along with LDL-C, whereas MUFA replacements reduced LDL-C while preserving HDL-C (207, 217). Studies trying to explain these results compared the different fatty acids to carbohydrate and found that each had HDL-C raising effects relative to carbohydrate, with PUFAs having the least and SFA having the greatest with MUFA closely following (218, 219). Further investigations demonstrated that the decreases in HDL-C and apoA1 were associated with increased fractional clearance of apoA1, which was later supported by further evidence showing decreased HDL-C was associated with reduced apoA1 production in addition to increased apoA1 clearance (220, 221). Therefore it is possible that MUFA may raise HDL-C by increasing secretion of apoA1 or reducing its clearance.

Investigation into the kinetics of HDL-C and apoA1 suggest possible mechanisms by which these levels are affected. These began as a result of many negative correlations observed clinically between plasma TG levels, HDL-TG content and fasting plasma HDL-C and apoA1 concentrations (222-226). Studies have demonstrated that the metabolism of HDL-C is affected by the interactions between TG-rich lipoproteins and HDL-C in plasma in at least two ways; firstly, through the lipolytic enzyme lipoprotein lipase (LPL) which breaks down TG-rich lipoproteins forming surface materials transferred to HDL, impacting its maturation (227-229), and secondly, through the cholesterol ester transfer protein (CETP) which transfers TGs from TG-rich lipoproteins to HDL in exchange for cholesterol esters (CE) producing TG-rich, CE-poor HDL which is more prone to catabolism (230-232). Kinetic studies in humans with hypertriglycerideridemia and low HDL-C have demonstrated that there is a significant increase in the fractional catabolic rate of apoA1 but no reduction in apoA1 production rates, therefore it is the enhanced clearance of HDL-C and apoA1 rather than a reduced production of HDL-C resulting from LPL which may contribute to the reduced
concentration of HDL-C (233-237). However, authors of these studies note that the CETP-mediated lipid exchange process itself can reduce plasma HDL-C but must be coupled with additional processes in order to reduce apoA1 concentrations which include the following: 1. TG-rich, CE-poor HDL are thermodynamically less stable and have the apoA1 more loosely bound, 2. TG-rich, CE-poor HDL are more readily lipolyzed by hepatic lipase (HL), which reduces HDL size and releases apoA1, 3. smaller HDL are more readily cleared from circulation (Figure 4). Since CETP is highly dependent on the concentration of TG-rich lipoproteins in the plasma, it is possible that by decreasing the concentration of TG in the circulation through decreasing glucose or carbohydrate intake by replacement of MUFA, HDL-C and apoA1 concentrations can be preserved by the converse of this proposed mechanism.

![Figure 4: Design of the proposed mechanism of HDL-C lowering (238).](image)

Therefore, increased MUFA consumption by replacement of carbohydrate, may decrease oxidative stress directly through free radical quenching as supported in studies where less oxidation is observed when SFA is replaced by MUFA compared to replacement by PUFA, or indirectly through reductions in glucose fluctuations and thus TG production, potentially preserving HDL-C along with its antioxidative properties.
3. Hypothesis, Objectives, and Rationale
3.1 Hypothesis
Increasing the dietary intake of MUFA will increase levels of HDL-C compared to the lower fat, higher carbohydrate diet, and decrease oxidative stress through reduced ox-LDL and increased protein thiols indicative of a protection of plasma proteins.

3.2 Objectives
Overall Objective: To assess the effect on serum lipids and oxidative damage to proteins and lipids of the replacement of carbohydrate with MUFA in a cholesterol-lowering diet.
1. To determine the effect of a cholesterol-lowering dietary portfolio combined with either high- or low-MUFA on HDL-C and apoA1.
2. To determine the effect of a cholesterol-lowering dietary portfolio in combination with either a high- or low-MUFA on oxidative damage to protein and lipids, as assessed by protein thiols and CD and TBARS in the LDL fraction, respectively.
3. To determine the effect of a cholesterol-lowering dietary portfolio combined with either high- or low-MUFA on LDL-C and apoB.
4. To determine the effect of a cholesterol-lowering dietary portfolio combined with either high- or low-MUFA on the total:HDL-C ratio and the apoB:A1 ratio.

3.3 Rationale
The dietary portfolio is a combination of foods which have been individually researched as effective in blood lipid control and to have some antioxidant activity. Previous dietary portfolio studies have consistently demonstrated significant reductions in LDL-C (10, 198, 199, 239). Recently, there is increasing concern in the risk factors for CVD other than LDL-C due to the residual risk that remains once LDL-C has been effectively reduced. One such risk factor includes low HDL-C which is present in over a third of adult men and women. Studies have suggested that for every 0.026mmol/L increase in HDL-C, there is a corresponding 1-3% decrease in CVD risk (53-55, 97).
There is much evidence that both MUFA and HDL-C have antioxidative potential and that the former may increase the later (13, 66, 71, 203, 218, 219, 240, 241). MUFAs can easily be incorporated into the diet through the use of high-MUFA oils including olive and canola oil, as well as avocados, nuts and seeds.

It therefore is appropriate to combine a high-MUFA background diet to an already effective cholesterol-lowering diet to further reduce the risk of CVD.
4. **Study: The Effect of Adding Monounsaturated Fat to a Dietary Portfolio of Cholesterol-Lowering Foods in Hypercholesterolemia**
The Effect of Adding Monounsaturated Fat to a Dietary Portfolio of Cholesterol-Lowering Foods in Hypercholesterolemia

4.1 Abstract

**Background**: Higher monounsaturated fat intakes may raise HDL-C without raising LDL-C. We have therefore tested whether increasing the monounsaturated fat (MUFA) content of an effective LDL-C lowering diet (dietary portfolio) both increases HDL-C and further reduces the total:HDL-C ratio, two key risk factors for CVD.

**Methods**: Twenty-four hyperlipidemic participants took a very low-saturated-fat therapeutic diet for one month and then were randomized to a low- or high-monounsaturated-fat dietary portfolio for a further month. Food was provided for the 2 month period with calorie intake based on Harris-Benedict estimates for energy requirement.

**Results**: On the high-MUFA dietary portfolio, HDL-C rose 13.1±3.6% (P=.004), whereas on the low-MUFA dietary portfolio, HDL-C did not change (2.7±3.6%, P=.466) (treatment difference, P=.004). The respective figures for the total:HDL-C ratio were -24.5±2.1% (P<.001) on high-MUFA versus -17.6±3.0% (P<.001) on low-MUFA (treatment difference, P=.006). These treatment differences were associated with significantly higher apoA1 concentrations on high-MUFA dietary portfolio. CRP was also significantly reduced on the high-MUFA dietary portfolio. No treatment difference was seen in body weight although a mean weight loss was seen over both low-MUFA (-0.98 ± 0.26kg) and high-MUFA (-0.78 ± 0.21kg) diets.

**Conclusions**: Monounsaturated fat increased the effectiveness of a cholesterol-lowering dietary portfolio and may reduce cardiovascular risk through raising HDL-C, further lowering the ratio of total:HDL-C and also by reducing CRP.
4.2 INTRODUCTION

Strategies that combine cholesterol-lowering foods or food components such as viscous fibres and plant sterols have been recommended to enhance the effectiveness of therapeutic diets low in saturated-fat and cholesterol (19, 242). Such dietary combinations (dietary portfolio) have resulted in substantial reductions in LDL-C (10) and its apolipoprotein, apoB, but the effects on HDL-C and its apolipoprotein, apoA1, have been less apparent (199). Low plasma HDL-C and apoA1 concentrations and an elevated ratio of total:HDL-C are recognized risk factors for CVD (243-247). Thus, dietary strategies that both lower total and LDL-C and raise HDL-C should have broad application. One method for increasing HDL-C appears to be the use of monounsaturated fat (MUFA), particularly when MUFA replaces dietary carbohydrates (248, 249). Furthermore, increased intakes of MUFA through increased nut consumption and vegetable oil intake has been associated with a reduced incidence of CVD in cohort studies (250, 251).

We have therefore compared the effect on serum lipids of substituting 13.0% of total calories as carbohydrate for MUFA in a dietary portfolio that has been shown to be effective under controlled conditions in lowering LDL-C by 28% and the total:HDL-C ratio by 24% (10).

4.3 METHODS

4.3.1 Participants

Men and women with mild to moderate hypercholesterolemia were recruited from the Clinical Nutrition and Risk Factor Modification Centre at St Michael’s Hospital, Toronto, Ontario, and from newspaper advertisements. Only postmenopausal women were recruited because of the increase in LDL-C and CHD risk in women of this age and to avoid possible fluctuations in blood lipids related to the menstrual cycle. Participants were selected who had previously demonstrated LDL-C levels >4.1 mmol/L (19, 242). No participants were recruited if they had a history of cardiovascular disease, untreated hypertension (blood pressure >140/90mmHg), diabetes, or renal or liver disease, or were taking medications known to influence serum lipids apart from stable doses of thyroxine. All medications and supplements were expected to be taken at a
constant dose prior to and during the study. Iron supplementation, ferrous gluconate 7mg tid (Salus-Haus, Bruckmuhl, Germany), was provided to participants on the basis of a prestudy ferritin of <50mcg/L.

4.3.2 Study Protocol

The study followed a randomized parallel design and was carried out between August 2007 and April 2009. Participants were counselled to follow their own low-saturated-fat therapeutic diets for 1 month prior to the start of the study. They were then provided with a very low-saturated-fat dairy and whole-grain cereal metabolically controlled diet which acted as a stabilization period prior to the high- and low-MUFA diets. After one month on this metabolically-controlled diet, they were randomized to take either a high-MUFA or conventional (low-MUFA) dietary portfolio for a final month.

Throughout the study participants attended weekly clinic visits where body weight was taken and blood pressure was measured three times in the non-dominant arm using an automated digital blood pressure monitor (OMRON Healthcare Inc, Vernon Hills, Illinois) by the same observer. At 2-week intervals blood samples were obtained after 12-hour overnight fasts. A seven-day diet history was obtained for the week prior to the 2-month metabolic treatment period. Completed menu checklists were returned at weekly intervals, as well as 7-day exercise records, during the 4-week diet periods and checked by the dietitians, who also checked the participants’ exercise and ensured that it was constant over the course of the study period. Exercise was calculated as metabolic equivalent of tasks (METs) (252). At weekly intervals, participants recorded their overall feeling of satiety using a 9-point bipolar semantic scale in which −4 was extremely hungry, 0 was neutral, and +4 was uncomfortably satiated.

Participants were randomized to the low- and high-MUFA portfolio diets and stratified on the basis of sex and an LDL-C of <4.2mmol/L or >4.2mmol/L during the first 2 weeks of the 4 weeks of the very low-saturated-fat therapeutic control diet by the statistician using a random number generator and SAS version 9.1 software (SAS Institute Inc, Cary, NC) (253) in a separate location from the clinic. The dietitians were not blinded to the diet because they were responsible for patients’ diets and for checking diet records. The laboratory staff responsible for analyses were blinded to
treatment and received samples labelled with name codes and dates. The study was approved by the ethics committees of the University of Toronto, St. Michael’s Hospital and the Natural Health Products Directorate of Health Canada. Written informed consent was obtained from all participants. Participants were offered no financial compensation for participation in the study. The clinical trial registration number is NCT00430430.

4.3.3 Diets

The diets eaten before the 8-week study were the participants’ routine therapeutic low-fat diets, which were similar to current National Cholesterol Education Program guidelines (<7% energy from saturated fat and <200 mg/d of dietary cholesterol) (19) and previously referred to as a Step II diet (248) (Table 2). During the 8-week study period, diets were provided based on estimated caloric requirements using the Harris-Benedict equation. Foods were sourced from Loblaws supermarket in Toronto and health food stores. All diets were vegetarian. In the case of the high-MUFA diet, 13.0% dietary calories as carbohydrate was replaced by MUFA in the dietary portfolio. MUFA was given in the form of a high-MUFA sunflower oil which contained 80% MUFA. Participants were also given the option to substitute a portion of the high-MUFA oil with a calculated amount of avocado. The aim of the dietary portfolio was to provide 1.0 g of plant sterols per 1000 kcal of diet in a plant sterol ester–enriched margarine (Flora Pro-Activ, Unilever, London, England) with a minimum of 2g/d and a maximum of 3g/d of plant sterols. The diet also provided 10.3 g of viscous fibres per 1000 kcal of diet from oats, barley, and psyllium; 20 g of soy protein per 1000 kcal as soy milk, tofu and soy meat analogs; and 21.5 g of whole almonds per 1000 kcal of diet. Emphasis was placed on eggplant and okra as additional sources of viscous fibre (0.2 g/1000 kcal and 0.4 g/1000 kcal, respectively). Eggs (2/wk) were also provided in the dietary portfolio to balance the saturated fat and dietary cholesterol in the very low-saturated-fat therapeutic control diet (Table 3). This dietary portfolio has been described in detail previously (10).

The very low-saturated-fat therapeutic control diet as eaten in the first month used skim milk, fat-free cheese and yogurt, and egg substitute and liquid egg white to
achieve a low intake of saturated fat. High fibre intake was obtained by the use of wholegrain breakfast cereals (fibre, 2.5g/1000 kcal of diet) and bread (fibre, 3g/1000 kcal of diet) made from 100% whole wheat flour. This diet therefore lacked sources of viscous fibres, plant sterols, soy protein and nuts. The macronutrient profile of the diet recorded as consumed over the 4 weeks achieved a saturated fat intake of <5% of total calories with <50mg cholesterol/1000kcal (Table 3).

Participants were provided with self-tarring electronic scales (Tanita Corporations, Arlington Heights, USA) and asked to weigh all food items consumed prior to and during the study period. During the study period, all foods to be consumed by participants were provided initially by courier and then at weekly clinic visits, with the exception of fruit and low calorie, non–starch-containing vegetables (8% and 24% of dietary calories respectively from high- and low-MUFA diets). Okra was the exception and was provided for the dietary portfolio. Participants were instructed to obtain specific fruit and vegetables from their local stores and were reimbursed on presentation of receipts. Participants were provided with 7-day rotating menus (Table 4). The diets have been described in detail previously (10).

Dietary compliance was assessed from the completed weekly checklists and from the return of uneaten food items.

4.3.4 Analyses

All serum samples from a given individual were labelled by code and analyzed in the same batch. Serum was analyzed according to the Lipid Research Clinics protocol (254) for total cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDL-C) by detergent solubilization and measurement of HDL-C (Roche Hitachi 917; Roche Diagnostics, Laval, Quebec, Canada), in the J. Alick Little Lipid Research Laboratory. Low-density lipoprotein cholesterol (LDL-C) was calculated (255). Serum apoA1 and apoB were measured by nephelometry, which is a measurement of scattered light by a dilute suspension of small particles (Dade BehringBNProSpec; Dade Behring Canada Inc, Mississauga, Ontario) (intra-assay coefficient of variation, 2.2% and 1.9%, respectively) (256). Serum samples, stored at −70°C, were analyzed for hs-CRP by end-point nephelometry (coefficient of variation, 3.5%) (Behring BN-100, N high-
sensitivity C-reactive protein reagent, Dade- Behring, Mississauga, Ontario). Diets were analyzed using a program based on US Department of Agriculture data (257) and developed in our laboratory to allow addition of data on foods relevant to ongoing studies after analysis in the laboratory for protein, total fat and dietary fibre using American Organization of Analytical Chemists methods and fatty acids by gas chromatography (10).

4.3.5 Statistical Analysis

Results were calculated as mean±SE. The study was an efficacy study with HDL-C as the primary outcome in the direct comparison of high- and low-MUFA dietary portfolio treatments. Since no within treatment difference was seen between weeks 2 and 4 and between weeks 6 and 8, the significance of the differences between treatments was assessed by the CONTRAST statement in SAS using all post baseline values (SAS PROC GLM) (258). The analysis used the change from baseline to weeks 2 and 4 for the very low-saturated-fat therapeutic control. The same weeks 2 and 4 of the control diet, in turn, acted as the baseline for the high- and low-MUFA dietary portfolios with weeks 6 and 8 as end of treatment response variables. Treatment, sex and their interaction were main effects, with baseline (mean of control weeks 2 and 4 in the portfolio analyses) as covariate. A 2-tailed paired t test was used to assess the significance of the within treatment (weeks 2 and 4) percentage change from baseline for the control and between weeks 2 and 4 (averaged) versus weeks 6 and 8 (averaged) for the high- and low-MUFA dietary portfolio groups. Means of weeks 2 and 4 data and weeks 6 and 8 data are presented in text and Tables.

With 12 participants per treatment group, and assuming an 8% SD of effect with α=.05 and 1-β=.80, we had sufficient power to detect a 9.6% change in HDL-C between treatments as significant.

4.4 RESULTS

4.4.1 Participants

Twenty-four healthy, hyperlipidemic participants were randomized to either the high- or low-MUFA arms of the study (17 men and 7 postmenopausal women) (Figure
Their baseline characteristics were similar (Table 1). Six participants had been taking statins and, after obtaining approval from their primary care provider, discontinued them as required by the study protocol at least 2 weeks prior to the study (3 low-MUFA and 3 high-MUFA dietary portfolio participants).

When expressed as the percentage of prescribed calories recorded as eaten over the 4 week period, compliance was high for all 3 diets: 93±2% for the very low-saturated-fat therapeutic control, 95±2% for the high-MUFA, and 91±2% for the low-MUFA dietary portfolio. At the end of each treatment all participants believed they were eating as much food as they were capable without experiencing discomfort (rating >3.0). Satiety ratings for treatments were: control, 2.1±0.2; high-MUFA dietary portfolio, 2.3±0.3; and low-MUFA dietary portfolio, 2.0±0.3. Participants lost a similar amount of weight on all 3 one-month treatments (control, -1.10±0.2kg; P<.001; high-MUFA dietary portfolio, -0.78±0.21kg; P=.003; low-MUFA dietary portfolio, -0.98±0.26kg; P=.003).

4.4.2 Blood Lipids and C-Reactive Protein

For the stabilization control diet, no treatment differences in baseline or weeks 2 and 4 blood measurements were observed between the 2 treatment groups (Table 5) who would subsequently be randomized to either high- or low-MUFA Portfolio diets (Figures 2 and 3). The mean percentage change from baseline using the data for the 24 participants was -15.6±1.4% (P<.001) for total-C, -21.5±2.0% (P<.001) for LDL-C, and 7.9±2.7% (P=.008) for HDL-C. The percentage changes were also significant for the total:HDL-C ratio (-21.9±1.9%, P<.001), apoA1 (3.7±1.6%, P=.028), apoB (-17.6±1.5%, P<.001) and the apoB:A1 ratio (-20.4±1.9%, P<.001). However the change in TG was not significant (-12.0±6.4%, P=.073).

For the dietary portfolio comparisons (Table 6), there were significant increases on high-MUFA but not low-MUFA in HDL-C, 13.1±3.6% versus 2.7±3.6% (P=.002); and apoA1, 7.8±2.2% versus -0.4±1.5% (P=.001). There were also significant reductions on high- versus low-MUFA in total:HDL-C, -24.5±2.1% versus -17.6±3.0% (P=.006); and apoB:A1, -23.2±1.6% versus -17.6±2.3% (P=.013). Similarly, hs-CRP was reduced on the high-MUFA, -37.5±10.6% but not on the low-MUFA dietary portfolio 45.3±33.4% (P=.003) (Figures 2 and 3).
There were no between treatment differences in LDL-C or apoB. However, significant within treatment reductions were seen on both high- and low-MUFA for LDL-C, $-20.9\pm2.6\%$ ($P<.001$) versus $-22.1\pm3.1\%$ ($P<.001$) and apoB $-17.2\pm2.1\%$ ($P<.001$) versus $-18.0\pm2.2\%$ ($P<.001$).

### 4.4.3 Blood Pressure

No significant treatment difference was observed in blood pressure (Tables 5 and 6).

### 4.4.4 Drop outs and Adverse Events

No participant withdrew from the study after the randomization which occurred during the first and second week of the control phase. One participant withdrew at the time of the first control breakfast, and therefore prior to randomization, due to the perceived inconvenience of a metabolically controlled diet. No adverse events related to the study protocol were reported.

### 4.5 DISCUSSION

The present study demonstrated that replacement of 13.0% of total calories from carbohydrate by MUFA in the dietary portfolio, while not altering the LDL-C reduction, resulted in a 10.4% greater increase in HDL-C over the 4 weeks. There was also a 6.9% greater reduction in the atherogenic index, total:HDL-C (247, 259) when compared to the low-MUFA dietary portfolio. This increase in HDL-C is of similar magnitude to two gemfibrozil studies which raised HDL-C concentrations by 6% and 8% and resulted in reductions in the relative risk of CHD of 22% and 23%, respectively (260, 261). The addition of MUFA by increasing HDL-C may therefore further enhance the cardioprotective effect of the cholesterol-lowering dietary portfolio.

The lines of evidence for a cardioprotective effect for HDL-C include epidemiologic and especially cohort studies (262-264). Measurements in the Framingham cohort have resulted in inclusion of the total:HDL-C ratio as the lipid parameter in the widely used Framingham CHD risk predictive equation (247) and post-hoc analysis of statin trials have identified HDL-C as predictive of major cardiovascular events even in participants whose LDL-C levels were optimized with a statin (243).
Intervention trials using gemfibrozil have also supported a cardioprotective role for raising HDL-C (261, 265), as have studies of apoA1 infusion, the apolipoprotein of HDL, which has been associated with plaque regression documented with intravascular ultrasound measurements (88, 266). These data together with laboratory studies (267, 268) have supported the concept that HDL is involved in reverse cholesterol transport, anti-oxidant and anti-inflammatory activities indicative of cardioprotective effects (97, 98).

Consequently, much emphasis has also been placed recently on lifestyle strategies to raise HDL-C including exercise (16, 17), weight loss (16, 17), moderate alcohol intake (16, 17), smoking cessation (16, 17), and increased monounsaturated fatty acid intake (17, 99, 208, 269). The increased MUFA intake is the only strategy relevant to the present study since participants were instructed to hold exercise constant, weight loss was similar in all treatments, alcohol intake was very small (a deviation from the dietary protocol but constant over both treatments), and no participant smoked.

Although some reservations have been expressed over the use of monounsaturated fats in preventing arteriosclerosis (270), previous studies have also noted the advantages of monounsaturated fat on the blood lipid profile, especially in type 2 diabetes, which is characterized by raised triglyceride and low HDL-C concentrations (271). Early studies showed that glycemic control was improved, serum triglycerides reduced and HDL-C increased with 25% substitution of carbohydrate by MUFA (215). Later studies have shown more variable results using a 15% calorie switch from carbohydrate to MUFA with reductions in VLDL-cholesterol and triglyceride but no change in HDL-C (272-274). In the present study, triglyceride was significantly reduced on the high- MUFA diet compared with the control diet but the treatment difference was not significant. The higher HDL-C and apoA1 on the high-MUFA compared to the low-MUFA diet resulted in significant reductions in the total:HDL-C and apoB:A1 ratios. These changes would be expected to reduce the risk for CHD (247).

The mechanisms by which MUFAs increase HDL-C are likely to be several. They include the ability of MUFA to scavenge free radicals (203, 275), reduce adipose tissue synthesis of pro-inflammatory cytokines and the stimulus to hepatic acute phase protein
synthesis, such as CRP. This metabolic environment would also allow increased synthesis of negative acute phase proteins, such as HDL, as seen in the present study. Our data are consistent with such a hypothesis, since plasma CRP concentration was reduced with the high-MUFA dietary portfolio. In addition, the displacement of dietary carbohydrates by MUFA is likely to result in less carbohydrate induced hepatic VLDL-TG synthesis. The resulting lower, VLDL-TG concentration, although not significant in the present study, may attenuate the impact of cholesterol-ester transfer protein (CETP) in depleting cholesterol ester of HDL, thereby contributing to a rise in plasma HDL-C concentration. The resulting TG-poor cholesterol-ester-rich HDL would also be predicted to be less rapidly lost from the circulation by tissue uptake than a TG-rich cholesterol-ester-poor particle and this is consistent with the observed increase in apoA1 concentrations after the high-MUFA dietary portfolio.

The limitations of the study include the relatively small subject numbers, weight loss, and the prescriptive nature of the diet, since adherence to the diet may be considerably less when eaten as a self-selected diet under real-world conditions. Despite these concerns, the effect size of the measurements of specific interest, HDL-C and apoA1 and their ratios, were substantial. In our post-hoc analysis, although our effect size for HDL-C was somewhat larger than expected, the standard deviation of effect was also larger and resulted in only 50% power. Nevertheless, the apolipoprotein of HDL-C, apoA1, with an effect size of 8.2% and a standard deviation of 6.6%, provided 83% power to detect the effect size as significant in favour of MUFA. Even though there was weight loss on all treatments, the loss of weight was similar and furthermore, did not influence the outcomes when included as a covariate in the statistical model. In terms of metabolically controlled conditions of the study, it is true that when the dietary portfolio has been consumed under self-selected real-world conditions, a mean adherence to the prescribed diet of only 64% was observed at 1 year. On the other hand, the high degree of adherence (95 and 91%) recorded on the present metabolic diets (low- and high-MUFA respectively) has allowed treatment differences to be more clearly demonstrated with relatively small subject numbers.
It should also be emphasized that the participants were largely non-obese (15/24), generally physically active, non-smokers on very low-saturated-fat diets. The MUFA increase therefore represented an addition to an otherwise healthy lifestyle and does not address the issue of the increased use of MUFA by those whose diet and lifestyles may be suboptimal (270). It would therefore be of great interest to test how addition of MUFA to an effective LDL-C lowering dietary portfolio can further increase plasma HDL-C in individuals with low levels such as those with the metabolic syndrome and type 2 diabetes.

The strengths of the study include the large effect size and the dietary metabolic control that ensured the uniform and very specific exchange of carbohydrate for MUFA in the diet. The study was also unique in determining the effect of adding MUFA to a diet which already had powerful cholesterol-lowering ability due to the very low-saturated-fat content and the use of nuts, viscous fibres (279), plant sterols (168, 171) and soy products (126, 128). The present study therefore demonstrated that MUFA preserved the HDL-C and apoA1 concentrations and so significantly increased the therapeutic potential of this dietary approach (246, 260-262, 280).

The total reductions in LDL-C and the total: HDL-C ratio on both the high- and low-MUFA dietary portfolio treatments which plateaued after 2 weeks were as large as any reported in previous Portfolio studies (Figures 2 and 3) (10). Expressed as the reduction from the start of the metabolic diets and including the reductions on both the therapeutic control diet and the further decrease on the high- or low-MUFA dietary portfolios, the total LDL-C reductions were 35.0% and 35.2% respectively. These LDL-C reductions are similar to those recorded in many of the earlier statin trials which resulted in 30-35% reductions in CHD risk (281).

At present, although there are no guidelines that specifically recommend raising HDL-C, the NCEP ATP III have a cut point for HDL-C of ≥1.0mmol/L with cut points for the metabolic syndrome of >1.0mmol/L for men and >1.3mmol/L for women (7) and the 2009 Canadian Cardiovascular Society Guidelines stratify cardiovascular risk for therapeutic purposes based partially on total: HDL-C ratio of >4 for high, >5 for medium, and >6 for low risk (18).
In conclusion, the CHD risk reduction potential of an effective cholesterol-lowering diet may be significantly enhanced by inclusion of a moderate amount of monounsaturated fat.
Figure 1: Patient flow diagram. *Chose not to participate (n=12): medical issue arose (n=4), want to lose weight (n=2), not willing to go off statin (n=1), not willing to stop vitamin supplements (n=1), not willing to give blood (n=1), not willing to do kinetics test (n=1), family issue (n=1), not interested (n=1).
Figure 2. Mean (SE) percentage change from baseline for both treatments for HDL-C, high-density lipoprotein cholesterol (P=0.004); apoA1, apolipoprotein A1 (P=0.001); total:HDL-C, total-cholesterol to high-density lipoprotein cholesterol ratio (P=0.045); apoB:A1, apolipoprotein B to apolipoprotein A1 ratio (P=0.030) (significance of difference between treatments) using the CONTRAST statement.
Figure 3. Mean (SE) percentage change from baseline for both treatments for total-C, total cholesterol (P=0.517); LDL-C, low-density lipoprotein cholesterol (P=0.473); apoB, apolipoprotein B (P=0.786); TG, triglyceride (P=0.538) (significance of difference between treatments) using the CONTRAST statement.
<table>
<thead>
<tr>
<th></th>
<th>High MUFA (n=12)</th>
<th>Low MUFA (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>Range</td>
</tr>
<tr>
<td>Age (y)</td>
<td>55 ± 7</td>
<td>42 - 68</td>
</tr>
<tr>
<td>Sex (female/male)</td>
<td>3/8</td>
<td>8/4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>83 ± 4</td>
<td>62 - 113</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29 ± 4</td>
<td>25 - 36</td>
</tr>
<tr>
<td>Total-C (mmol/L)</td>
<td>6.3 ± 0.2</td>
<td>5.4 - 7.7</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>4.3 ± 0.2</td>
<td>3.5 - 5.6</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.14 ± 0.08</td>
<td>0.73 - 1.66</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.9 ± 0.2</td>
<td>1.0 - 3.5</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>127 ± 3</td>
<td>109 - 142</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>77 ± 3</td>
<td>57 - 91</td>
</tr>
<tr>
<td>Exercise, METs</td>
<td>21.7 ± 5.2</td>
<td>1.5 - 55.0</td>
</tr>
<tr>
<td>Medications, No. of subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Pressure</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Thyroxine (0.08mg OD)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Aspirin 81mg</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Supplements:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron Supplementation (7mg TID)</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Multivitamin</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Calcium</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Glucosamine Chondritin</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Discontinued prior to study*</td>
<td>n=3</td>
<td>n=3</td>
</tr>
<tr>
<td>Statins:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crestor 5mg</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Crestor 10mg</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Crestor 20mg</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Lipitor 20mg</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

† Data are expressed as mean±SE. No significant baseline differences were observed between the high and low-MUFA groups.
* participants taking statins were asked to discontinue them 2 weeks prior to beginning the study

Abbreviations: MUFA, monounsaturated fatty acid, BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; METs, metabolic equivalent of tasks (an index number expressing the energy cost of physical activities as multiples of Resting Metabolic Rate); SI conversion factors: To convert cholesterol and triglycerides to mg/dL, divide by 0.0259 and 0.0113, respectively.
<table>
<thead>
<tr>
<th></th>
<th>Habitual Diet:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High MUFA Group</td>
<td>Low MUFA Group</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n=12)</td>
<td>(n=12)</td>
<td></td>
</tr>
<tr>
<td>Calories (kcal)</td>
<td>1882</td>
<td>1914</td>
<td></td>
</tr>
<tr>
<td>Protein (%)</td>
<td>16.6</td>
<td>15.7</td>
<td></td>
</tr>
<tr>
<td>Soy Protein (%)</td>
<td>0.0</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Available CH₂O (%)</td>
<td>57.3</td>
<td>56.1</td>
<td></td>
</tr>
<tr>
<td>Dietary Fiber (g/1000kcal)</td>
<td>18.6</td>
<td>17.5</td>
<td></td>
</tr>
<tr>
<td>Viscous Fiber (g/1000kcal)</td>
<td>2.0</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>Fat (%)</td>
<td>24.0</td>
<td>28.2</td>
<td></td>
</tr>
<tr>
<td>SFA (%)</td>
<td>6.1</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>MUFA (%)</td>
<td>9.6</td>
<td>11.5</td>
<td></td>
</tr>
<tr>
<td>PUFA (%)</td>
<td>5.4</td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td>Dietary Cholesterol (mg/1000kcal)</td>
<td>61.6</td>
<td>60.6</td>
<td></td>
</tr>
<tr>
<td>Alcohol (%)</td>
<td>2.1</td>
<td>1.5</td>
<td></td>
</tr>
</tbody>
</table>

*Data are expressed as mean percentage of calories per day unless otherwise noted. Abbreviations: CH₂O, carbohydrate; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid*
<table>
<thead>
<tr>
<th></th>
<th>NCEP Control Diet (n=24)</th>
<th>Low MUFA Portfolio (n=12)</th>
<th>High MUFA Portfolio (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories (kcal)</td>
<td>2442&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2345&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2536&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>20.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soy Protein (%)</td>
<td>0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Available CH₂O (%)</td>
<td>51.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.9&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dietary Fiber (g/1000kcal)</td>
<td>20.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Viscous Fiber (g/1000kcal)</td>
<td>0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>27.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>45.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>SFA (%)</td>
<td>4.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MUFA (%)</td>
<td>10.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.9&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>PUFA (%)</td>
<td>9.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dietary Cholesterol (mg/1000kcal)</td>
<td>38.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.8&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alcohol (%)</td>
<td>0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.38&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Data are expressed as mean percentage of calories per day unless otherwise noted where different letters as superscripts on the same line indicate significant differences between treatments (P<0.05) as assessed by ANCOVA.

Abbreviations: CH₂O, carbohydrate; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; NCEP Control Diet, National Cholesterol Education Program-based Control Diet.
Table 4: Example Menuplan of Diets for 2000kcal

<table>
<thead>
<tr>
<th>Control Diet</th>
<th>High MUFA Diet</th>
<th>Low MUFA Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Breakfast:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cereal:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bran flakes cereal*</td>
<td>30 grams</td>
<td>Out bran</td>
</tr>
<tr>
<td>Skim milk</td>
<td>220 grams</td>
<td>Unsweetened Soy Beverage</td>
</tr>
<tr>
<td><strong>Fruit&amp;Yogurt:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strawberries</td>
<td>100 grams</td>
<td>Strawberries</td>
</tr>
<tr>
<td>Fat Free Yogurt*</td>
<td>175 grams</td>
<td>Psyllium (+ water 250ml)</td>
</tr>
<tr>
<td>Strawberry Jam*</td>
<td>10 grams</td>
<td>Toast: (Bread, Oat bran)</td>
</tr>
<tr>
<td><strong>Drink:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skim milk</td>
<td>220 grams</td>
<td>Psyllium (+ water 250ml)</td>
</tr>
<tr>
<td><strong>Snack:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pear</td>
<td>133 grams</td>
<td>Almonds</td>
</tr>
<tr>
<td>Skim milk</td>
<td>220 grams</td>
<td>Pear</td>
</tr>
<tr>
<td><strong>Lunch:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pasta &amp; Fagioli Soup*</td>
<td>270 grams</td>
<td>Barley Vegetable Soup*</td>
</tr>
<tr>
<td>Whole wheat bread</td>
<td>90 grams</td>
<td>Oat bran bread</td>
</tr>
<tr>
<td>Fat Free Cheese</td>
<td>42 grams</td>
<td>Study margarine (Plant sterol-enriched)</td>
</tr>
<tr>
<td>Salad: Lettuce, Romaine</td>
<td>30 grams</td>
<td>Lettuce, Romaine</td>
</tr>
<tr>
<td>Cucumber</td>
<td>50 grams</td>
<td>Cucumber</td>
</tr>
<tr>
<td>Tomato</td>
<td>100 grams</td>
<td>Tomato</td>
</tr>
<tr>
<td>Oil, Olive</td>
<td>11 grams</td>
<td>Almonds (chopped)</td>
</tr>
<tr>
<td>Vinegar</td>
<td>10 grams</td>
<td>High-MUFA Sunflower Oil</td>
</tr>
<tr>
<td><strong>Snack:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ancient Grain Crackers*</td>
<td>10 grams</td>
<td>Almonds</td>
</tr>
<tr>
<td>Orange</td>
<td>133 grams</td>
<td>Apple</td>
</tr>
<tr>
<td><strong>Dinner:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheese Cannelloni*</td>
<td>246 grams</td>
<td>Low-Fat Extra-Firm Tofu*</td>
</tr>
<tr>
<td>Bake/grill: Okra</td>
<td>100 grams</td>
<td>Okra</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>100 grams</td>
<td>Cauliflower</td>
</tr>
<tr>
<td>Onions</td>
<td>30 grams</td>
<td>Onions</td>
</tr>
<tr>
<td>Green pepper</td>
<td>50 grams</td>
<td>Green pepper</td>
</tr>
<tr>
<td>Fat Free Cheese</td>
<td>42 grams</td>
<td>High-MUFA Sunflower Oil</td>
</tr>
<tr>
<td><strong>Snack:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skim milk</td>
<td>220 grams</td>
<td>Unsweetened Soy Beverage</td>
</tr>
<tr>
<td>Apple</td>
<td>133 grams</td>
<td>Psyllium (+ water 250ml)</td>
</tr>
</tbody>
</table>

*These products were Blue Menu line products (Loblaws, Toronto, Canada)
Table 5. Effect of Control, very low saturated fat diet, on Blood Lipids, C-Reactive Protein, and Blood Pressure in High- vs. Low-MUFA Groups

<table>
<thead>
<tr>
<th></th>
<th>Control prior to HighMUFA (n=12)</th>
<th>Control prior to LowMUFA (n=12)</th>
<th>P-value£</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 2&amp;4</td>
<td>Difference (SE)</td>
</tr>
<tr>
<td>Body Weight, kg</td>
<td>83.1 (4.1)</td>
<td>82.4 (4.1)</td>
<td>- 0.7 (0.3)*</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total-C</td>
<td>6.33 (0.20)</td>
<td>5.48 (0.20)</td>
<td>- 0.85 (0.13)‡</td>
</tr>
<tr>
<td>LDL-C</td>
<td>4.34 (0.20)</td>
<td>3.67 (0.17)</td>
<td>- 0.67 (0.11)‡</td>
</tr>
<tr>
<td>HDL-C</td>
<td>1.14 (0.08)</td>
<td>1.00 (0.05)</td>
<td>- 0.14 (0.10)†</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.87 (0.21)</td>
<td>1.77 (0.12)</td>
<td>- 0.10 (0.17)</td>
</tr>
<tr>
<td>Apolipoproteins, g/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ApoA1</td>
<td>1.43 (0.05)</td>
<td>1.28 (0.05)</td>
<td>- 0.14 (0.03)‡</td>
</tr>
<tr>
<td>ApoB</td>
<td>1.23 (0.05)</td>
<td>1.10 (0.04)</td>
<td>- 0.13 (0.02)‡</td>
</tr>
<tr>
<td>Ratios</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total:HDL-C</td>
<td>5.64 (0.40)</td>
<td>5.63 (0.29)</td>
<td>- 0.21 (0.17)</td>
</tr>
<tr>
<td>LDL:HDL-C</td>
<td>4.01 (0.31)</td>
<td>3.78 (0.23)</td>
<td>- 0.23 (0.10)*</td>
</tr>
<tr>
<td>ApoB:A1</td>
<td>0.88 (0.05)</td>
<td>0.87 (0.05)</td>
<td>- 0.01 (0.01)</td>
</tr>
<tr>
<td>hs-CRP, mg/L</td>
<td>1.17 (0.25)</td>
<td>1.62 (0.24)</td>
<td>0.44 (0.25)</td>
</tr>
<tr>
<td>Blood Pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>126.6 (3.0)</td>
<td>120.5 (2.4)</td>
<td>- 6.1 (2.2)*</td>
</tr>
<tr>
<td>Diastolic</td>
<td>76.6 (2.8)</td>
<td>73.6 (2.3)</td>
<td>- 3.0 (1.5)</td>
</tr>
</tbody>
</table>

Abbreviations: MUFA, monounsaturated fatty acid; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; hs-CRP, high-sensitivity C-reactive protein

* significantly different from Week 0 at P<0.05, † at P<0.01, ‡ at P<0.001;
£ difference between changes in high- versus low-MUFA groups using the CONTRAST statement

To convert total-C, LDL-C, and HDL-C to mg/dL, divide by 0.0259; for triglycerides to mg/dL, divide by 0.0113; for ApoA1 and B to mg/dL, multiply by 100.
<table>
<thead>
<tr>
<th></th>
<th>High MUFA Portfolio (n=12)</th>
<th>Low MUFA Portfolio (n=12)</th>
<th>P-value£</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 2&amp;4</td>
<td>Week 6&amp;8</td>
<td>Difference (SE)</td>
</tr>
<tr>
<td>Body Weight, kg</td>
<td>82.4 (4.1)</td>
<td>81.6 (4.0)</td>
<td>- 0.78 (0.21)†</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>5.48 (0.20)</td>
<td>4.66 (0.09)</td>
<td>- 0.82 (0.13)‡</td>
</tr>
<tr>
<td></td>
<td>3.67 (0.17)</td>
<td>2.89 (0.13)</td>
<td>- 0.78 (0.11)‡</td>
</tr>
<tr>
<td></td>
<td>1.00 (0.05)</td>
<td>1.13 (0.07)</td>
<td>0.13 (0.03)†</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.77 (0.12)</td>
<td>1.41 (0.09)</td>
<td>- 0.36 (0.07)‡</td>
</tr>
<tr>
<td>Apolipoproteins, g/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>1.28 (0.05)</td>
<td>1.38 (0.06)</td>
<td>0.10 (0.03)†</td>
</tr>
<tr>
<td>B</td>
<td>1.10 (0.04)</td>
<td>0.90 (0.03)</td>
<td>- 0.19 (0.03)‡</td>
</tr>
<tr>
<td>Ratios</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total:LDL-C</td>
<td>5.63 (0.29)</td>
<td>4.21 (0.18)</td>
<td>- 1.42 (0.17)‡</td>
</tr>
<tr>
<td>LDL:HDL-C</td>
<td>3.78 (0.23)</td>
<td>2.62 (0.14)</td>
<td>- 1.16 (0.12)‡</td>
</tr>
<tr>
<td>ApoB:A1</td>
<td>0.87 (0.05)</td>
<td>0.67 (0.03)</td>
<td>- 0.21 (0.02)‡</td>
</tr>
<tr>
<td>hs-CRP, mg/L</td>
<td>1.62 (0.24)</td>
<td>0.82 (0.13)</td>
<td>- 0.79 (0.21)†</td>
</tr>
<tr>
<td>Blood Pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>120.6 (2.4)</td>
<td>119.6 (2.1)</td>
<td>- 1.00 (2.52)</td>
</tr>
<tr>
<td>Diastolic</td>
<td>73.5 (2.3)</td>
<td>72.5 (2.2)</td>
<td>- 0.96 (1.36)</td>
</tr>
</tbody>
</table>

Abbreviations: MUFA, monounsaturated fatty acid; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; hs-CRP, high-sensitivity C-reactive protein.

* significantly different from Week 2&4 at P<0.05, † at P<0.01, ‡ at P<0.001;
£ difference between changes in high- versus low-MUFA groups using the CONTRAST statement.

To convert total-C, LDL-C, and HDL-C to mg/dL, divide by 0.0259; for triglycerides to mg/dL, divide by 0.0113; for ApoA1 and B to mg/dL, multiply by 100.
5. The Effect on Oxidative Stress of Adding Monounsaturated Fat to a Dietary Portfolio of Cholesterol-Lowering Foods
5.1 Abstract

**Background**: Higher monounsaturated fat (MUFA) intakes may reduce oxidative damage and contribute to reduced cardiovascular disease (CVD) risk by decreased oxidative damage.

**Methods**: Twenty-four hyperlipidemic subjects took a very low-saturated-fat therapeutic control diet for one month and then either a high- or low-MUFA dietary portfolio for a further month. Food was provided for the 2-month period based on Harris-Benedict estimates for requirement. Oxidative stress was measured as serum protein thiols and conjugated dienes (CD) and thiobarbituric acid reactive substances (TBARS) in the LDL fraction.

**Results**: Although all treatments showed antioxidant activity, there were no significant differences between the high- and low-MUFA dietary portfolios in any of the measures of oxidative damage.. For the group as a whole (24 subjects), during the control diet there were significant increases in protein thiols of 7.6 ± 3.1% (P=0.023) and significant reductions in CD of 9.3 ± 3.0% (P=0.005) but non-significant reductions in TBARS of 8.5 ± 5.8% (P=0.154). During both the high- and low-MUFA dietary portfolios, protein thiols increased significantly by 8.8 ± 3.7% (P=0.026) while CD and TBARS decreased significantly by 9.6 ± 3.5% (P=0.012) and 8.5 ± 3.5% (P=0.021) respectively, indicating a reduction in oxidative damage.

**Conclusions**: No effect of MUFA could be detected, although all diets showed antioxidant activity.
5.2 INTRODUCTION

Evidence suggests that dyslipidemia and oxidative stress are key mechanisms associated with the development of atherosclerosis and cardiovascular disease (CVD) (282). Dyslipidemia has been closely associated with increased endothelial production of reactive oxygen species (ROS) and the oxidative modification of low-density lipoprotein (LDL)-associated lipids which is directly involved in the initiation of atherosclerotic development (282). The dietary portfolio appears to reduce CVD risk through reduction of both LDL-C and C-reactive Protein (CRP) (10, 11). Oxidized LDL (ox-LDL), a further risk factor, may also be reduced because of the nature of the key components of the dietary portfolio (12). Reductions in ox-LDL have been demonstrated in studies involving soy protein or almonds (114, 115, 119, 120, 145, 146) and in some studies on plant sterols and fibres, although the data are not as strong (165, 184, 185, 187). It was therefore of interest to determine whether the addition of monounsaturated fatty acids (MUFAs) to the dietary portfolio provided a further benefit in terms of antioxidant since MUFA consumption has been shown to reduce oxidative modification of LDL (203, 240, 283).

We have therefore compared the effect on oxidative stress of substituting 13.0% of total calories as MUFA for carbohydrate in an effective cholesterol-lowering dietary portfolio.

5.3 METHODS

5.3.1 Participants

Men and post-menopausal women with mild to moderate hypercholesterolemia were recruited and completed the study (17 men and 7 postmenopausal women). Their mean ± SE age was 55 ± 9 years (range, 38-69 years) and mean body mass index (BMI) was 28.8 ± 0.7 kg/m² (range, 24.4-36.0 kg/m²) (refer to Table 1 in Chapter 4). All participants had previously demonstrated high LDL-C levels >4.1 mmol/L (19, 242). No participants had a history of cardiovascular disease, untreated hypertension (blood pressure >140/90mmHg), diabetes, or renal or liver disease, and none were taking medications known to influence serum lipids apart from 1 woman taking stable doses of thyroxine. Six participants were taking statins and had discontinued them as required at
least 2 weeks prior to the study (3 low-MUFA and 3 high-MUFA dietary portfolio participants). All medications and supplements were expected to be taken at a constant dose prior to and during the study. Five participants were taking antihypertensive medications at a constant dose prior to and during the study and 4 were taking aspirin or other anti-inflammatory drugs. Other, more commonly used non-prescription drugs and supplements taken throughout the study period included a daily multivitamin (n = 4), calcium (n = 8), glucosamine (n=2), melatonin (n=2) and vitamin D (n=5). Iron supplementation (ferrous gluconate 7mg tid) was provided to 16 subjects on the basis of a prestudy ferritin of <50mcg/L.

5.3.2 Study Protocol

The study followed a randomized parallel design and was carried out between August 2007 and April 2009. Participants followed their own low-saturated-fat therapeutic diet for 1 month prior to the start of the study. They were then placed on a very low-saturated-fat dairy and whole-grain cereal diet which acted as a stabilization period prior to the high- and low-MUFA diets. After one month on this metabolically-controlled diet, they were randomized to either a high-MUFA or conventional (low-MUFA) dietary portfolio for a final month. All foods were provided except for fresh fruit and vegetables which subjects were prescribed and instructed to obtain for themselves.

Throughout the study participants attended weekly clinic visits where body weight was taken and blood pressure was measured three times in the non-dominant arm using a mercury sphygmomanometer by the same observer. At 2-week intervals blood samples were obtained after 12-hour overnight fasts. A seven-day diet history was obtained for the week prior to the 2-month metabolic treatment period. Completed menu checklists were returned at weekly intervals during the 4-week diet periods and checked by the dietitians, who also recorded the participants’ previous week’s exercise and ensured that it was constant over the course of the study period. At weekly intervals, participants recorded their overall feeling of satiety using a 9 point bipolar semantic scale in which −4 was extremely hungry, 0 was neutral, and +4 was uncomfortably satiated.
The statistician stratified the participants on the basis of sex and an LDL-C of 4.2mmol/L in the first 2 weeks of the very low-saturated-fat therapeutic control diet using a random number generator and SAS version 9.1 software (SAS Institute Inc, Cary, NC) in a separate location from the clinic. The dietitians were not blinded to the diet because they were responsible for patients’ diets and for checking diet records. The laboratory staff responsible for analyses were blinded to treatment and received samples labelled with name codes and dates. The study was approved by the ethics committees of the University of Toronto and St. Michael's Hospital and the Natural Health Products Directorate of Health Canada. Written informed consent was obtained from all participants. The clinical trial registration number is NCT00430430.

5.3.3 Diets

The diets eaten before the 8-week study were the participants’ routine therapeutic low-fat diets, which were similar to current National Cholesterol Education program guidelines (<7% energy from saturated fat and <200 mg/d of dietary cholesterol) (19, 284) and previously referred to as a Step II diet (248). During the 8-week study period, diets were provided based on estimated caloric requirements by the Harris-Benedict equation and all diets were vegetarian. In the case of the high-MUFA diet, 14.7% dietary calories as starch was replaced by MUFA in the dietary portfolio. The aim of the dietary portfolio was the same as previous dietary portfolio studies (10, 198): to provide 1.0 g of plant sterols per 1000 kcal of diet in a plant sterol ester–enriched margarine with a minimum of 2.0g and a maximum of 3.0g of plant sterols per day; 10.3 g of viscous fibres per 1000 kcal of diet from oats, barley, and psyllium; 20 g of soy protein per 1000 kcal as soy milk and soy meat analogs; and 21.5 g of whole almonds per 1000 kcal of diet. Eggs (2/wk) were provided in the dietary portfolio to balance the saturated fat and dietary cholesterol in the very low-saturated-fat therapeutic control diet (refer to Table 4 in Chapter 4). The very low-saturated-fat therapeutic control diet has been previously described (refer to Chapter 4). The macronutrient profile of the diet recorded as consumed over the 4 weeks achieved a saturated fat intake of <5% of total calories with <50mg cholesterol/1000kcal.
Participants were provided with self-tarring electronic scales (Tanita Corporations, Arlington Heights, USA) and asked to weigh all food items consumed prior to and during the study period. During the study period, all foods to be consumed by participants were provided initially by courier and then at weekly clinic visits, with the exception of fruit and low-calorie, non–starch-containing vegetables (8% and 24% of dietary calories respectively from high- and low-MUFA diets). Okra was the exception and was provided in the dietary portfolio. Participants were provided with 7-day rotating menus (refer to Table 3 in Chapter 4).

Compliance was assessed from the completed weekly checklists and from the return of uneaten food items.

5.3.4 Analyses

Serum lipids were measured on samples stores at -70°C in the same batch according to the Lipid Research Clinics protocol (254) for total-C, TG, and HDL-C after dextran sulphate–magnesium chloride precipitation (285) and LDL-C was calculated (255). Serum apolipoprotein A1 and B were measured by nephelometry (intra-assay coefficient of variation, 2.2% and 1.9%, respectively) (256) and hs-CRP by end-point nephelometry (coefficient of variation, 3.5%) (Behring BN-100, N high-sensitivity C-reactive protein reagent, Dade- Behring, Mississauga, Ontario).

All samples from a given individual were labelled by code and analyzed in the same batch. Protein oxidation was measured using the 5,5′-dithiobis (2-nitrobenzoic acid) assay (38) to assess the loss of reduced thiol (−SH) groups as a measure of oxidation. The coefficient of variation of serum samples analyzed in triplicate was 3.0%. LDL oxidation was estimated by measuring in the LDL fraction both CD, based on the method of Ahotupa et al (39), and TBARS, using the malondialdehyde (MDA)–thiobarbituric acid assay adopted from Jentzsch et al (40). The coefficients of variation for these analyses were 5.7% and 6.4% for TBARS and CD, respectively.

Diets were analyzed using a program based on US Department of Agriculture data and developed in our laboratory to allow addition of data on foods relevant to ongoing studies after analysis in the laboratory for protein, total fat, and dietary fibre
using American Organization of Analytical Chemists methods and fatty acids by gas chromatography (10).

5.3.5 Statistical Analysis

Results were calculated as mean±SE. The significance of the differences between the treatments for the oxidative markers were assessed using ANOVA in SAS (258) with the change from baseline to week 4 for the very low-saturated-fat therapeutic control diet and from week 4 to week 8 for the high- and low-MUFA dietary portfolios. Treatment and sex and their interaction were main effects, with baseline as covariate. A 2-tailed paired t test was used to assess the significance of the within treatment (weeks 0 and 4) percentage change from baseline for the control and between week 0 versus week 8 for the high- and low-MUFA dietary portfolio groups.

The original power calculation for the study was based on the ability to detect changes in lipids and lipoproteins (refer to Chapter 4). In the present analysis, with the observed SD of effect of 16.9% for TBARS and 12 subjects per group, we could be able to detect a 20.2% difference in the primary measure (α=0.05, 1- β=0.8); for CD, with the observed SD of effect of 17.6% for CD, we could be able to detect a 21.0% difference in CD between the 2 treatments; and similarly for protein thiols, with the observed SD of effect of 18.5%, we could be able to detect a 22.1% difference.

5.4 RESULTS

5.4.1 Participants

No significant differences were found in baseline data between the 2 groups, both diets were similarly well complied with (>90%), there was no difference between satiety ratings between the 2 groups, and participants lost a similar amount of weight on all 3 one-month treatments (control, -1.10 ± 0.2kg; P<.001; high-MUFA dietary portfolio, -0.78 ± 0.21kg; P=.003; low-MUFA dietary portfolio, -0.98 ± 0.26kg; P=.003).

5.4.2 Markers of Oxidation

No differences were observed among the 2 treatment groups in baseline oxidative measurements and no differences were seen between the high- and low-
MUFA diets. Increases in protein thiols and reductions in CD and TBARS were observed throughout the study on both high- and low-MUFA diets (Tables 1 and 2). The mean percentage change over the control month using the data for the 24 subjects for protein thiols was $7.6 \pm 3.1\%$ ($P=0.023$) and for CD and TBARS was $9.3 \pm 3.0\%$ ($P=0.005$) and $8.5 \pm 5.8\%$ ($P=0.154$), respectively. For the 24 subjects during the portfolio diets, protein thiols further increased significantly by $8.8 \pm 3.7\%$ ($P=0.026$) while CD and TBARS further decreased significantly by $9.6 \pm 3.5\%$ ($P=0.012$) and $8.5 \pm 3.5\%$ ($P=0.021$) respectively, indicating a reduction in oxidative damage (Figure 1). In post-hoc analyses, there were no differences in oxidative measures between subjects when they were divided into those receiving iron supplementation or those who lost weight (data not shown).

5.4.3 Drop out and Adverse Events

No participant withdrew from the study after the randomization which occurred during the first and second week of the control phase. One participant withdrew at the time of the first control breakfast due to the perceived inconvenience of a metabolically controlled diet. No adverse events were reported.

5.5 DISCUSSION

This study demonstrated that replacement of 13.0% carbohydrate by MUFA resulted in no differences between treatments for the oxidative markers protein thiols, CD or TBARS. However, the antioxidant activity of the very low-saturated-fat therapeutic control diet and both the high- and low-MUFA dietary portfolios were reflected in the progressive increase in protein thiols and reduction in conjugated dienes and TBARS and therefore present a potential reduction in LDL atherogenicity (22, 30, 31).

In the present study, the very low-saturated-fat control diet can be seen as a positive control since low-fat dairy diets emphasizing fruits and vegetables, such as in the Dietary Approaches to Stop Hypertension (DASH) trial, have previously demonstrated reductions in markers of oxidation, as well as improved serum lipid profiles (50). The continued improvement in the oxidative measures during the second
month of the study in which the subjects were consuming the dietary portfolio is anticipated since many of the key components, including plant sterols, soy protein, viscous fibres and almonds, have previously demonstrated the potential to reduce oxidative stress such as reductions in ox-LDL (119, 145, 146, 183, 185), thus suggesting the potential to protect LDL from oxidative damage (147, 148). Additionally, almonds have demonstrated increases in total antioxidant capacity (120) and their skins have shown antioxidant activity against free radicals in humans (121, 122) while whole grains have been associated with reductions in risk of inflammatory diseases (166). Therefore the dietary portfolio contains a variety of foods which may explain the observed reduction in oxidative markers.

Although studies adding MUFA to a diet have demonstrated a reduction in oxidative damage, such as reductions in the oxidative modification of LDL particles (203), no difference between the high- and low-MUFA treatments were observed in the present study. However, in the low-MUFA diet, MUFA was replaced by carbohydrates which came from fruits, which resulted in a diet containing around 24% of its dietary calories from fruit and vegetable as opposed to the 8% in the high-MUFA diet. Therefore, since MUFA was replaced by fruits which are known to be rich in antioxidants, it may be that there was no significant difference between the high- and low-MUFA diets because both diets were rich in antioxidant foods of a different nature. For example, some studies have demonstrated an approximate 10-15% reduction in CDs on a high-MUFA versus low-MUFA diet over 1 month, (203, 286), while the DASH (Dietary Approaches to Stop Hypertension) diet which contained about 16% of calories from fruits and vegetables demonstrated a 10% reduction in urinary isoprostanes, a marker of oxidative damage. Therefore, whether from increased MUFA or from a diet rich in fruits and vegetables, there were similar reductions in markers of oxidative damage. These reductions are similar to those observed in the present study where there were reductions of 8.5% in CDs on the DASH-like control diet and another 10% during the high-MUFA dietary portfolio. The further 10% reduction in CDs on the low-MUFA dietary portfolio may be the result of the added fruit as well as the dietary portfolio components.
Additionally, due to the inclusion of multiple foods with antioxidant potential including a healthy DASH-like control diet, MUFAs, and fruit and vegetables, a possible reason as to why no additional improvement in oxidative markers resulted from the high-MUFA treatment is that the dietary benefit may have already been maximal since some ability to mount an oxidative response is required for normal functioning including defence by white cells from invading bacteria (287).

However, it is also possible that we did not see treatment differences in the oxidative measures because of the relatively small sample size of 12.

The present study demonstrates that therapeutic diets rich in fruit, vegetables, soy, nuts and other antioxidant components result in a reduction in oxidative damage. No treatment difference was seen when MUFA was included in the diet possibly due to the displacement of other antioxidant dietary components. Furthermore, because of the high level of natural antioxidants, no effect of iron status was observed in this relatively small group of subjects. More extreme situations in which there is a lack of antioxidant activity provided by the control diet may have to be employed to demonstrate the effect of dietary antioxidants.
Figure 1. Means of oxidative stress on high \( [n=12, \blacktriangle] \) and low \( [n=12, \blacklozenge] \) MUFA diets. 1 outlier was removed from the high-MUFA group in the analysis of CD (127.2, 102.3, 125.0 at Week 0, 4, 8, resp.). 1 outlier was removed from the low-MUFA group in the analysis of protein thiols (200.4 at week 8) and of CD (95.6 at week 8) and another outlier in the analysis of TBARS (2.7, 1.5, 1.4 at week 0, 4, 8, resp.).
<table>
<thead>
<tr>
<th></th>
<th>Control-Low-MUFA (n=12)</th>
<th>Control-High-MUFA (n=12)</th>
<th>High vs. Low Group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protein Thiols (mmol/L)</strong></td>
<td>328 ± 21</td>
<td>347 ± 20</td>
<td>20 ± 12</td>
</tr>
<tr>
<td><strong>Conjugated Dienes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute Concentration (umol)</td>
<td>65 ± 6</td>
<td>60 ± 6.4</td>
<td>- 5 ± 3</td>
</tr>
<tr>
<td>Molar ratio of LDL-C (umol/mmol)</td>
<td>15.0 ± 1.5</td>
<td>16.0 ± 1.7</td>
<td>1.0 ± 0.8</td>
</tr>
<tr>
<td><strong>TBARS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute Concentration (umol)</td>
<td>1.28 ± 0.15</td>
<td>1.03 ± 0.07</td>
<td>- 0.25 ± 0.10*</td>
</tr>
<tr>
<td>Molar ratio of LDL-C (umol/mmol)</td>
<td>0.29 ± 0.04</td>
<td>0.27 ± 0.02</td>
<td>- 0.02 ± 0.02</td>
</tr>
</tbody>
</table>

* significantly different from Week 0 (P<.05)

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<table>
<thead>
<tr>
<th></th>
<th>Low MUFA Portfolio (n=12)</th>
<th>High MUFA Portfolio (n=12)</th>
<th>High vs. Low</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protein Thiols (mmol/L)</strong></td>
<td>347 ± 20</td>
<td>376 ± 23</td>
<td>29 ± 15</td>
</tr>
<tr>
<td><strong>Conjugated Dienes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute Concentration (umol)</td>
<td>60 ± 6</td>
<td>53 ± 5</td>
<td>- 7 ± 4</td>
</tr>
<tr>
<td>Molar ratio of LDL-C (umol/mmol)</td>
<td>16.0 ± 1.7</td>
<td>17.5 ± 1.6</td>
<td>1.6 ± 1.0</td>
</tr>
<tr>
<td><strong>TBARS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute Concentration (umol)</td>
<td>1.03 ± 0.07</td>
<td>0.96 ± 0.06</td>
<td>- 0.07 ± 0.05</td>
</tr>
<tr>
<td>Molar ratio of LDL-C (umol/mmol)</td>
<td>0.27 ± 0.02</td>
<td>0.32 ± 0.02</td>
<td>0.05 ± 0.02*</td>
</tr>
</tbody>
</table>

* significantly different from Week 4 (P<.05)
6. Overall Discussion and Limitations
6.1 Overall Discussion

Dietary modification has been shown to reduce risk of CVD (19, 269) which explains the continuous search for better dietary strategies to target multiple risk factors. Just as 4 foods that had been individually shown to reduce cholesterol demonstrated an additive effect when combined to form the dietary portfolio, MUFA was added in an effort to further reduce the overall risk for CVD. As recent studies demonstrate, there is a residual risk that remains after effective LDL-C reduction. Therefore, strategies to target this residual risk are of great interest.

In this study, the high-MUFA treatment was able to effectively raise HDL-C significantly more than the low-MUFA treatment. This is an important result since studies indicate that there is a 1-3% associated reduction in risk of CVD for every 0.026mmol/L increase in HDL-C (53-55, 57). Additionally, the lipid results of the high-MUFA treatment demonstrate that not only is HDL-C being improved, but also apoA1 levels, which may be an important indication of the quality of HDL since apoA1 has important roles associated with HDL-C. For example, in RCT apoA1 is the ligand for the ABC (ATP-binding cassette) transporter, and therefore is the docking point for which HDL can receive cholesterol from peripheral cells and transport it to the liver (96). It is also the cofactor for LCAT (lecithin cholesterol acyltransferase) which is important in removing cholesterol from tissues and transporting them via HDL to the liver (96). Also, this study demonstrates that in addition to the significant reduction in LDL-C, one of the possible factors contributing to residual CVD risk, HDL-C, was also improved thereby potentially providing further CVD risk reduction, especially for those who ~20% of people who attain low LDL-C but who may still experience a cardiovascular event (53, 288).

The significant increase in HDL-C is also an important finding of the study because it provides strong support for the idea that MUFA consumption can raise HDL-C, which has been largely debated. The present study provides strong evidence because of the following: it included a 4-week metabolically controlled run-in period (the low-saturated-fat control diet) which acted as a stabilization period for all subjects prior to the randomization to high- or low-MUFA portfolio diets; it was very well controlled, with all foods provided to the participants' homes, as well as reimbursement for any items purchased for the study diet, there were weekly clinic visits for counselling with
suggestions for new recipes and ideas on how to combine foods for each participants’ personal preferences, as well as to assist in any difficulties encountered; and it provided a moderate but significant amount of MUFA in the form of an enriched sunflower oil which could easily be consumed in daily food preparation. Therefore, it allowed for all subjects to attain comparable baseline values as a result of the stabilization period, provided the tools for successful adherence to the diets, and achieved a significant difference between treatments making them suitable for comparison.

The significant HDL-C increase on the high-MUFA diet is also promising because the other methods of raising HDL-C, such as through the use of fibrates or niacin therapy, have negative side effects making them undesirable. Niacin has been associated with flushing, dry skin, and gastrointestinal irritation, as well as increased risk of hepatotoxicity when taken as a monotherapy (17). Fibrates are less effective compared to niacin therapy in raising HDL-C, and may also increase serum creatinine and homocysteine levels and thus require close patient follow-up (17). Therefore, the results of this study are very appealing because the high-MUFA was able to raise HDL-C to a comparable level to that of the mentioned pharmacotherapies, allowing for a convenient and effective substitution in the diet without the undesired side effects.

The other beneficial observation in the present study which may provide further CVD risk reduction was the significant reduction in hs-CRP in the high-MUFA group compared to the low-MUFA group. Thus, added benefit may arise from the anti-inflammatory properties of HDL, or the result of apoA1 since evidence exists that the interaction between apoA1 and the ABC transporter can act as an anti-inflammatory receptor in a separate pathway from that in RCT (289). CRP is receiving greater attention due to the recently published JUPITER trial which demonstrated the lowest cardiovascular event rate was achieved in subjects with an LDL-C level less than 2.0mmol/L and an hs-CRP level less than 2.0mg/L (290). As a result of this trial, hs-CRP has been incorporated into recommended clinical guidelines, such as in the Canadian Cardiovascular Society Guidelines in which it is stated that in intermediate-risk groups with LDL-C levels which do not require treatment, hs-CRP is part of risk stratification in addition to its inclusion as a secondary target (18). Additionally, the total-HDL-C ratio is in the guidelines as a secondary target and is included as a specific therapy target in
those classified as intermediate risk (18). Many studies have also concluded that it is one of the best predictors of long-term risk (17, 18, 291, 292). In the present study, both hs-CRP and total:HDL-C ratio were significantly reduced in the high-MUFA group, thus there is the potential for MUFA to assist in improving these targets for CVD risk reduction. Some more recent studies suggest that the apoB:A1 ratio may be a better predictor of CVD risk than total:HDL-C ratio, however in the present study, the apoB:A1 ratio was also significantly reduced on the high-MUFA diet versus the low-MUFA diet, therefore the same dietary interventions would be appropriate to help reduce risk via this alternative ratio predictor (81).

Since LDL-C has a well established direct relationship with the development of atherosclerosis and its oxidation appears to accelerate this process, strategies to reduce oxidative stress are recognized as important (22, 30). Although no treatment differences were observed in markers of oxidative stress, the diets as a whole were antioxidant and may assist in CVD risk reduction. Firstly, both dietary portfolios reduced LDL-C by 20-22%, which was preceded by a 15.5% reduction on the stabilization control diet, therefore reducing the substrate itself in the atherosclerotic development process. Over the 2 month period, LDL-C was reduced by about 1.5mmol/L in both treatment groups which when applied to previously established and well recognized findings that 1.0mmol/L reduction in LDL-C is associated with a 21% reduction in CVD risk (20), these diets would be associated with a 31.5% reduction in risk of CVD. Secondly, irrespective of MUFA intake, there was a significant increase in protein thiols and significant reductions in CD and TBARS, which overall indicate reductions in oxidative damage both to proteins and lipids. Therefore, diets which are rich in whole grains and low-fat dairy, as in the control diet, may reduce oxidative damage and diets which also contain soy, nuts, and are rich in fruits and vegetables or MUFAs, as in the dietary portfolio treatments, may provide further reductions. Although there is controversy over the effects of antioxidants on oxidative damage, the majority of studies are looking at supplementation in which case you may not get the appropriate diversity of antioxidants required to observe expected benefits. In the present study, the control diet provided reductions due to a few foods with some potential effects which was followed by greater reductions seen on the dietary portfolio containing additional foods with potential benefit in addition to
either more fruit or high MUFAs. Thus, by providing an increment in variety of foods with antioxidant potential, the study was able to demonstrate continuous reductions in oxidative damage. Therefore, by using whole foods, there is a wider variety of components which may act together to elicit effects on oxidative damage and thus may contribute to reduction in CVD risk (293).

   Overall, through the combination of foods which lower cholesterol and oxidative damage, including the use of MUFAs, there is potential for greater reductions in CVD risk.

6.2 Limitations and Future Directions

   In considering the limitations of the study, the prescriptive nature of the diet must be addressed since the subjects received all their foods by courier and were given menuplans indicating all foods to be eaten and at what amounts. The dietary portfolio has been observed under real-world conditions with an adherence rate of 64% at 1-year. However, with the suggested inclusion of MUFA, MUFA must be encouraged not to displace the portfolio components but to replace other sources of fat in the diet and not to be incorporated as an addition to the existing diet, which could easily result in weight gain.

   Another limitation was the weight loss which occurred throughout the duration of the study period, as well as the relatively small sample size (n=24). The weight loss may have been the result of the use of the Harris-Benedict predictive equation for energy requirement which had quite a large activity factor that the present group of subjects generally fell just under, so may not have been prescribed amounts that accounted for their exercises. Also, they may not have actually been consuming the entire amount prescribed because of remaining amounts in the cooking pans, in transferring, or leftover on their plates. However, weight loss was consistent throughout the study and similar among both diet groups and did not influence any study outcomes when included as a covariate in the statistical model. In terms of our sample size, although small, we had enough power to detect the effect size in apoA1 as significant in favour of MUFA (α=0.05).
We may have been unable to detect differences in the oxidative markers between the high- and low-MUFA groups because the nature of the foods which MUFA replaced have been shown to have antioxidant activity, that is, fruit. Ideally, to be able to observe the true effects of MUFA on oxidative damage, the reference or control diet would have had little to no antioxidant effect. However, we wanted to compare the effect of adding MUFA to a dietary portfolio, which already contains potential antioxidant activity in its components. Also, in order to keep all the portfolio components at their prescribed levels, the only carbohydrate source which would be available for replacement was that from fruit. Therefore the reference diet, low-MUFA, was rich in foods also capable of antioxidant activity, albeit with an unknown comparability to the activity of MUFAs.

6.3 Future Research

Since there was no difference between the high- and low-MUFA dietary portfolios in the reduction in oxidative damage demonstrated in this study, it would be interesting to test other measures of oxidative damage given that similar studies have found differences although the carbohydrates replaced in those studies contained more simple sugars and low-fat dairy (203). This could include other measures of lipid oxidation, such as isoprostanes, and protein oxidation, such as protein carbonyls, as well as measures of oxidative damage to DNA, such as 8-hydroxydeoxyguanosine (8-OHdG) (294). It may also be interesting to test if the samples from the high-MUFA groups had greater antioxidant activity by such tests as the radical absorbance capacity (ORAC). For a more direct measure of HDL-C antioxidant activity, levels of PON-1 could be measured, since PON1 is the HDL enzyme component which some studies have reported to be responsible for the antioxidative activity of HDL (13, 74, 75). Additionally, a larger sample size in future studies will allow for greater power to detect differences in oxidative markers, as well as the selection of a reference treatment in which MUFA would replace carbohydrate sources without antioxidant activity in order to detect the activity of MUFAs.

HDL-C has also been recognized for its antithrombotic activities and studies done with recombinant HDL-C or apoA1 have demonstrated regression of plaques and improvements in atherosclerosis. Therefore, it would be interesting to conduct a larger
trial to try to raise HDL-C and apoA1 in subjects, for example with acute coronary syndrome, and to include such measurements as intravascular ultrasound (IVUS) as a measure of atheroma volume for which administration of recombinant apoA1Milano has demonstrated significant improvement (88). This would allow for detection of quality of HDL-C and functionality of apoA1 to see if raised apoA1 can reach the effectiveness seen in the mutated version, apoA1Milano. Other groups with low levels of HDL-C, such as in diabetes or the metabolic syndrome, both of which are also accompanied by elevations in triglyceride, would be potentially interesting groups to observe effects because of their increased risk of CVD (295).
7. Summary
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In carrying out this thesis the aim was to answer the objectives. We have demonstrated the following:

1. HDL-C and apoA1 were significantly increased on the high-MUFA versus the low-MUFA dietary portfolio without compromising the lipid-lowering effectiveness of the diet.

2. Although there was no treatment difference between the high- and low-MUFA dietary portfolio groups, in the subject group as a whole, there was a significant increase in protein thiols and reductions in both CD and TBARS in the LDL fraction, indicating reduced oxidative damage to serum proteins and lipids.

3. Both dietary portfolio treatments significantly reduced LDL-C and apoB irrespective of MUFA intake.

4. Total:HDL-C ratio, as well as apoB:A1 ratio were significantly reduced on the high-MUFA versus the low-MUFA dietary portfolio.
8. References


78. Tselepis AD, Elisaf M, Goudevenos J, et al. PAF-acetylhydrolase is an antiinflammatory and antioxidant phospholipase A2 that may be implicated in atherothrombosis. Atheroscler Suppl 1999;144:1.


in vivo assessment by intravascular ultrasound and magnetic resonance imaging. J Am Coll Cardiol 2008;51:1098-103.


166. Jacobs DR, Jr., Andersen LF, Blomhoff R. Whole-grain consumption is associated with a reduced risk of noncardiovascular, noncancer death attributed


223. Patsch JR, Prasad S, Gotto AM, Jr., Patsch W. High density lipoprotein2. Relationship of the plasma levels of this lipoprotein species to its composition, to the magnitude of postprandial lipemia, and to the activities of lipoprotein lipase and hepatic lipase. J Clin Invest 1987;80:341-7.


