# EVALUATION OF A FUNCTIONAL OIL COMPOSED OF MEDIUM CHAIN TRIACYLGLYCEROLS, PHYTOSTEROLS AND n-3 FATTY ACIDS ON THE CARDIOVASCULAR RISK PROFILE OF OVERWEIGHT WOMEN

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## ABSTRACT

We examined the effect of a functional oil (FctO), with potential weight-controlling and blood lipid-lowering attributes, vs beef tallow as control (C), on the cardiovascular risk profile of overweight women. The FctO comprised energy expenditure-enhancing medium chain triacylglycerols, cholesterol-lowering phytosterols and triacylglycerol-suppressing n-3 fatty acids. In a randomized, single-blind, crossover design, inpatient trial, 17 women consumed each oil as part of a controlled, supervised, energy-adjusted diet for 27 days. Body weight decreased similarly during both dietary periods. Plasma total and LDL cholesterol levels decreased by 4.8% and 10.4% following FctO, and were lower by 9.0% and 16.4 % respectively, after FctO vs C. HDL cholesterol and circulating triacylglycerol levels were unaffected by treatment, though HDL:LDL and HDL:total cholesterol ratios increased by 19.5% and 9.4% on FctO. Plasma total homocysteine levels were higher on FctO vs C. Plasma glutathione increased with FctO supplementation.

We conclude that consumption of FctO improves the overall cardiovascular risk profile of overweight women.

## RÉSUMÉ

Nous avons évalué l'effet d'une huile fonctionnelle (HFct), ayant des attributs potentiels sur le contrôle du poids corporel et la diminution des lipides sanguins, en comparaison à la graisse de bœuf comme contrôle (C), sur le profil cardiovasculaire des femmes ayant un surplus de poids. L'HFct était composée de triacylglycérols à chaînes moyennes, de phytostérols et d'acides gras n-3, ayant pour buts respectifs d'augmenter la dépense énergétique, de réduire le cholestérol sanguin et de diminuer les triglycérides sanguins. À fin de ne pas révéler l'identité des gras, ceux-ci ont été incorporés à une diète contrôlée qui contribuait un niveau d'énergie spécifique pour chaque individu. Dans un ordre arbitraire, 17 femmes ont consommé chaque diète sous supervision pendant 27 jours, durant lesquels elles résidaient de façon permanente au centre de recherche. Le poids corporel a diminué de façon similaire pendant les 2 phases alimentaires. L'HFct a diminué les taux de cholestérol total et LDL sanguins de 4.8% et 10.4%, respectivement. Les valeurs finales de cholestérol total et LDL étaient plus basses de 9.0% et 16.4%, respectivement, sur la diète HFct comparé à la diète C. Les concentrations du cholestérol HDL et des triacylglycérols plasmatiques sont demeurées inchangées suite au traitement. Les ratios de cholestérol HDL:LDL et HDL:total ont augmenté favorablement de 19.5% et 9.4% suite à la diète HFct. Les niveaux d'homocystéine totale sanguine étaient plus élevés sur la diète d'HFct, comparé à la diète C. L'HFct a augmenté les niveaux plasmatiques de l'antioxydant glutathion.

En conclusion, la consommation de l'HFct améliore le profil cardiovasculaire chez les femmes ayant un surplus de poids.

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## CONTRIBUTION OF AUTHORS

The first manuscript included as part of this thesis is entitled "*Health Claims on Foods in Canada: Toward Successful Implementation*" and provides an introduction to the area of functional foods and health claims, with respect to the developing health claim regulation in Canada. As the primary author, I elaborated the concepts and suggestions regarding health claim regulation included in this manuscript. I was responsible for writing and formatting the manuscript, as well as creating the figure and tables. As my supervisor, Peter Jones provided the general subject for the manuscript, as well as editorial assistance.

The second manuscript included as part of this thesis is entitled "*Consumption of a functional oil composed of medium chain triacylglycerols, phytosterols and n-3 fatty acids improves the overall cardiovascular risk profile of overweight women*" and reports the experimental part of the thesis. Contribution of authors to this manuscript follows.

As the first author, I was responsible for writing and formatting this manuscript, in addition to creating tables and figures. I was co-research coordinator for the human feeding trial described in this manuscript. I have been extensively involved with the execution of every aspect of this inpatient study, completed over an 8-month period. More specifically, I was responsible for subject recruitment, information session presentations, subject screening, selection and randomization, preparation of daily meals in the metabolic ward, and subject supervision at the nutrition unit during meals and between meal hours, including evenings and nights. Assistance in meal preparation and subject supervision was provided by Christopher Vanstone, Erika Motoie, Andrea D'Souza and Sarah Miller. I was also responsible for blood draw scheduling and processing of collected samples. Lucie Gravel performed blood draws on subjects and Catherine Vanstone coded blood sample tubes for blinding purposes. Christopher Vanstone coded blood tubes on weekend collection days. I performed all blood lipid measurements, including screening, and certification procedures on the Abbott Analyser. I also completed sample preparation and gas chromatography fatty acid analyses on food, red blood cells and fecal samples presented in this manuscript. In addition, I was responsible for all statistical analysis of the data included in the present manuscript.

Peter Jones, Jeffrey Cohn, Andrea Papamandjaris and Marie-Pierre St-Onge provided editorial assistance with the manuscript. Marie-Pierre St-Onge, as a co-research coordinator, was also involved in subject recruitment, feeding and supervision at the metabolic unit. She also designed the experimental diets. Christopher Vanstone and Ms St-Onge processed fecal samples to the freeze-dried state. Then, Mahmoud Raeini-Sarjaz, myself and Ms St-Onge performed lipid extractions on the freeze-dried samples, and I carried out the remaining saponification and derivatization procedures, as well as gas chromatography work on these samples alone. Plasma thiols were measured by Louis-Jacques Fortin at the Institut de Recherche Clinique de Montréal, in collaboration with Jeffrey Cohn. Andrea Papamandjaris and Peter Jones elaborated the original concept of the functional oil tested in this study, as well as the study design. They wrote grant proposals to obtain funding to conduct this human feeding trial; this was done

before I started graduate studies. As my supervisor and principal investigator of the project, Peter Jones provided direction to the study.

# 1. INTRODUCTION AND OVERVIEW

The role of good nutrition in improving health and preventing diseases was already known to early civilizations. Hippocrates, the father of modern medicine, expressed it in these terms: "*Let food be thy medicine and medicine be thy food" and " If we could give every individual the right amount of nourishment and exercise, not too little and not too much, we would have found the safest way to health*" (Hippocrates, 460-377 B.C.). Traditional Chinese medicine has always made use of active ingredients found in plants and other edible material to restore balance in body constituents and body forces. The advancement of science in recent years has allowed for elucidation of mechanisms responsible for disease development, and the role of specific biological substances, many of which naturally occur in foods, in mediating the progress of chronic conditions. As a consequence of improved nutritional knowledge, the concept of functional food has emerged. Functional foods are conventional foods, or foods modified by addition of a beneficial nutrient/bioactive substance or removal of a detrimental substance. They are consumed as part of a habitual diet and have physiological benefits, or reduce the risk of chronic disease, beyond basic nutritional functions (Health Canada, 1998). In order to respond to the growing interest in functional foods of consumers and food industries, and given the potential health benefits to the general population and resulting reduction in health care costs, health claims, which describe the relationship between a food/nutrient/biological substance and its effect on physiological functions or chronic disease risk reduction, have been added to food labels in some countries. In Canada, health claim regulation is still in the development and consultation phase. The first part of the present literature review describes the proposed framework for evaluating health claims on foods in Canada (Health Canada, June 2000) and discusses different

considerations for successful health claim implementation and functional food promotion. Consumer confidence in health claims, sustainable development of the functional food industry and effective communication of health claims to consumers are likely determinants of the success health claims can have in modifying eating behaviours. The second part of the literature review describes a specific functional food with potential health benefits to help reduce the risk of developing obesity and cardiovascular disease (CVD). This functional food has been tested for its effect on the cardiovascular risk profile of overweight women in the experimental work of this thesis.

Obesity constitutes a health risk for the development of several chronic conditions, including hyperinsulinemia, Type II diabetes, certain types of cancer, hyperlipidemia, hypertension, and CVD, the latter being the most common cause of mortality and morbidity in North America (Statistics Canada and Health Canada, 1997; National Institutes of Health, 1998). An experimental oil having weight-maintaining and lipid-lowering properties would thus be of benefit for reducing the risk of obesity and CVD, and would consequently offer considerable health benefits to Western societies. The present functional oil (FctO) contains 3 major components previously shown to have a positive health impact: medium chain triacylglycerols (MCT), phytosterols, and n-3 polyunsaturated fatty acids (PUFA).

Medium chain fatty acids (MCFA) are saturated fatty acids (FA) having 8, 10 or 12 carbons, and are naturally found in coconut oil, and in small amounts in butter. These shorter chain FA are also found in breast milk and are thought to play an important nutritional role in infant development (Giovannini et al, 1991). Extracted from coconut oil, MCT oil is composed of caprylic (8:0) and capric (10:0) acids, and benefits from the generally recognized as safe (GRAS) status in the United States. It is presently used as a

therapeutic tool for malabsorption disorders and is found as well in parenteral and enteral nutrition formulas (Bach and Babayan, 1982; Bach et al, 1996). Benefits of MCFA are ascribed to their reduced chain length, which results in differential absorption and metabolism, relative to long chain fatty acids (LCFA). The increased energy expenditure and fat oxidation following MCT consumption (Hill et al, 1989; Scalfi et al, 1991; Dulloo et al, 1996; White et al, 1999) has indicated a role for MCT in maintaining healthy body weights and in promoting weight loss. However, the benefits of MCT to energy balance may be counteracted by increases in circulating cholesterol and triacylglycerol (TAG) concentrations, which are risk factors contributing to the development of heart disease. We have therefore suggested the combination of MCT with cholesterol-lowering agents, such as phytosterols, and with TAG-lowering agents, such as n-3 PUFA, as a mean of using MCT in weight management while maintaining cardiovascular health.

Phytosterols naturally occur in the fat-soluble fractions of plant material, where they function in a similar fashion to their structurally similar animal counterpart, cholesterol. By blocking the absorption of cholesterol from the gut, phytosterols have been shown to lower plasma total and LDL cholesterol levels (Heinemann et al, 1986; Miettinen et al, 1995; Jones et al, 1999; Hendriks et al, 1999). Plant sterols are presently manufactured in functional foods such as margarines and salad dressings, under trade names like Benecol™ and TakeControl™ in Europe, the United States and other countries. A health claim for the role of plant sterols esters and its saturated derivative, stanols esters, was approved in the United States in September 2000 (Food and Drug Administration, 2000). Given that the cholesterol-lowering efficacy of phytosterols has already been established, their incorporation into the present FctO should improve the CVD profile.

n-3 Polyunsaturated fatty acids are found in marine organisms, such as fatty fishes, in the form of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The plant precursor to these long chain n-3 PUFA, alpha-linolenic acid (ALA), is found in large proportion in flaxseed, with more than half of constituent FA being ALA. Consumption of n-3 PUFA has been associated with decreased risk of CVD in Eskimos (Bang and Dyerberg, 1980) and is known to affect many aspects of heart disease development (Connor, 2000). Thus, n-3 PUFA provide multiple benefits for protection against CVD, including lowering of plasma TAG levels and possibly reducing of plasma homocysteine (Hcy) concentrations (Olszewski and McCully, 1993).

The FctO examined in this thesis combines the increased energy expenditure of MCT, the cholesterol-lowering abilities of phytosterols, and the hypotriacylglycerolemic qualities of n-3 PUFA. This functional food has been developed to help maintain healthy body weights and blood lipid levels, in the context of increased obesity prevalence and high mortality from CVD in Western societies.

# 2. LITERATURE REVIEW

## 2.1. Manuscript 1:

### Health Claims on Foods in Canada: Toward Successful Implementation

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**Running head**: Health claim regulation in Canada

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### 2.1.1. Case Presentation

While doing her weekly grocery shopping, a post-menopausal woman notices a new calcium-enriched orange juice with the following health claim:

> *"A healthy diet with adequate calcium and vitamin D, and regular physical activity, help to achieve strong bones and may reduce the risk of osteoporosis. This product is a good source of calcium."*
>
> (Canada Gazette Directorate, 2001).

The woman's thoughts are drawn to her mother who has had two hip fractures within the last year and is now immobilized in a nursing home for elderly, as a result of osteoporosis. She wonders if she should switch orange juice brand to increase her calcium intake and help prevent osteoporosis, especially since her family physician was recently concerned about her decreased bone density. She also thinks of her teenage daughter who, although minimally concerned about the impact of nutrition on her health, is in her critical years of calcium building bone. Could increasing her calcium intake by consuming products like calcium-enriched juice allow her to lead more satisfying retirement years? Could it allow her daughter to reverse her genetic predisposition to osteoporosis? In effect, how successful will Health Canada's initiative to implement health claims be in improving the health of Canadians?

### 2.1.2. Introduction

A growing body of scientific evidence substantiates the role of diet in maintaining health and helping to prevent certain risk-modifiable chronic diseases. Examples are

numerous, such as dietary fat type that modulates blood cholesterol levels and thus risk of cardiovascular disease (Hornstra et al, 1998). The carotenoid lycopene present in tomatoes appears to lower the risk of prostate cancer (Agarwal and Rao, 2000), while excess dietary sodium exacerbates hypertension in sensitive individuals (Weinberger, 2000). Preliminary evidence indicates that antioxidant compounds found in diverse fruits and vegetables may act to prevent oxidative damage to various tissues and reduce the risk of certain cancers (Craig, 1997). Foods containing such nutrients/bioactive substances in optimal amounts are called "functional foods", since their consumption may lead to health benefits. Health Canada currently proposes to define a functional food as "*similar in appearance to, or may be, a conventional food, consumed as part of a usual diet, and is demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions*" (Health Canada, 1998).

Communicating the role of diet, and of functional foods in particular, in health improvement and disease risk reduction to consumers is the main objective in establishing health claims. Assuming that behavioural changes could be achieved by health claim implementation, substantial benefits could potentially accrue in terms of reduced morbidity, mortality and health care expenses. A cost-benefit study, conducted by the Food and Drug Administration in the United States (Food and Drug Administration, 1993) on the potential impacts of changing food labeling following the establishment of the Nutrition Labeling and Education Act (NLEA), concluded that 12,600 lives and 21 billions US dollars could be saved over a 20-year period if consumers were to change their eating behavior. A more recent estimation by Agriculture and Agri-Food Canada and Health Canada (Canada Gazette Directorate, 2001) suggested that 5 billion dollars could be saved over the next 20 years in terms of reductions in the direct

and indirect costs resulting from cancer, diabetes, and coronary heart disease and stroke if Canadians were to also change their eating habits. This estimation reflects potential health benefits that could be achieved following a Health Canada initiative to improve nutrition information, by the revision of nutrition labeling and nutrient content claims, as well as the establishment of health claims.

Given the evidence for disease risk reduction following consumption of several bioactive substances in food, and the resulting benefits to improve health for the general population and to lower health care costs, Health Canada is developing a policy on health claims. The objectives of this paper is to describe Health Canada's "*Standards of evidence for evaluating foods with health claims: A proposed framework*" (Health Canada, June 2000), and to address three aspects of an effective health claim policy implementation: consumer confidence in health claims, sustainable development of the functional food industry, and effective communication of health messages to consumers. By doing so, we hope to raise awareness amongst health care professionals on this issue, and promote their implication in the health claim policy development process.

### 2.1.3. Health Claim Regulation in Canada

In order to respond to the growing interest in functional foods by consumers and food industries, a policy regarding health claims for foods was initiated in 1996 (Health Canada, Nov 2000), and after extensive consultations and discussions, the Food Directorate and the Therapeutic Products Programme of Health Canada published a policy decision in November of 1998 (Health Canada, 1998). This policy recommends that structure/function and risk reduction claims, being generic or product-specific, be

permitted and implemented under the current Food and Drug Act. A structure/function claim describes "*the role of a dietary ingredient to affect a structure or function in humans*" (Health Canada, 1998). An example would be the role of dietary calcium in maintaining healthy bones. A risk reduction claim describes the role of a nutrient in "*altering major risk factors involved in the development of a chronic disease or abnormal physiological condition*" (Health Canada, 1998), such as intake of sodium and risk of high blood pressure, an important risk factor for heart disease. A generic claim "*can be applied to any food or food product meeting the criteria of the claim*", while a product-specific claim "*is made for a single commercial product and cannot be generalized to other similar products unless acceptable supporting evidence is provided*" (Health Canada, 1998). Examples of risk reduction generic claims are provided in **Table 2.1.1**.

The first step in Health Canada's claim policy implementation is the development of regulatory amendments to permit diet-related generic health claims. This includes the review of health claims presently approved in the United States under the NLEA (Health Canada, Aug 2000), presented in Table 2.1.1 along with proposed wording for pre-approved claims determined to be scientifically valid by Health Canada. On June 16, 2001, a regulatory proposal for use of the 5 pre-approved diet-related health claims was published in the Canada Gazette Part I (Canada Gazette Directorate, 2001), and was part of a broader initiative to enhance nutrition information in labeling and advertising. Wording and compositional criteria are defined within this document, and comments were sought for a 90-day period, after which the final regulation would be published in the Gazette as Part II. The second aspect of the policy implementation is the development of standards of evidence for evaluating foods with health claims with the publication of

**Table 2.1.1**: Generic health claims considered as part of the Canadian health claim policy implementation [1]

| Pre-approved health claims and proposed wording | Health claims under evaluation by Health Canada |
|---|---|
| • A healthy diet containing foods high in potassium and low in sodium may reduce the risk of high blood pressure, a risk factor for stroke and heart disease. This food is a good source of potassium and is sodium-free. [2]<br>• A healthy diet with adequate calcium and vitamin D, and regular physical activity, help to achieve strong bones and may reduce the risk of osteoporosis. This food is an excellent source of calcium. [2]<br>• A healthy diet low in saturated and trans fats may reduce the risk of heart disease. This food is free/low in saturated and trans fats.<br>• A healthy diet rich in a variety of fruits and vegetables may help reduce the risk of some types of cancer.<br>• Tooth friendly. Does not promote dental caries. | • Folate and neural tube defects<br>• Fibre-containing grain products, fruits and vegetables and cancer<br>• Fruits, vegetables and grain products that contain fibre, particularly soluble fibre and risk of coronary heart disease |

[1] Obtained from: Consultation document on generic health claims (Health Canada, Aug 2000) and Regulations amending the food and drug regulations (Canada Gazette Directorate, 2001).

[2] Different nutrient contents are possible for these health claims.

the proposed framework in June 2000 (Health Canada, June 2000), later described in this paper. Comments and feedback on the proposed framework were sought from several sources, including consumer groups, industries, academia, and the general public, prior to September 2000 (Canada Gazette Directorate, 2001). These inputs, as well as results of testing consumer perception of health claims, will guide the development of a final framework for evaluating health claims, as well as a guidance document for submitting claim requisitions. The third aspect, which still remains to be addressed, is the development of a regulatory framework to permit product-specific health claims on foods.

### 2.1.4. Standards of Evidence for Evaluating Foods with Health Claims

The proposed framework published in June 2000 (Health Canada, June 2000) describes requirements in product safety, claim validity and quality assurance that would be used to evaluate foods with health claims, as represented in **Figure 2.1.1**. Information required to evaluate these three aspects of health claims are summarized in **Table 2.1.2**.

#### *2.1.4.1. Product Safety*

Consumption of new functional foods with added or removed dietary components affecting health, as well as changes in conventional food consumption patterns, would result in modified exposure to several bioactive substances. Therefore, Health Canada considers that high standards of evidence are required for evaluating the safety of foods bearing health claims. Adverse health effects must be minimized since food products are destined for unrestricted consumption by the general population. As part of the proposed

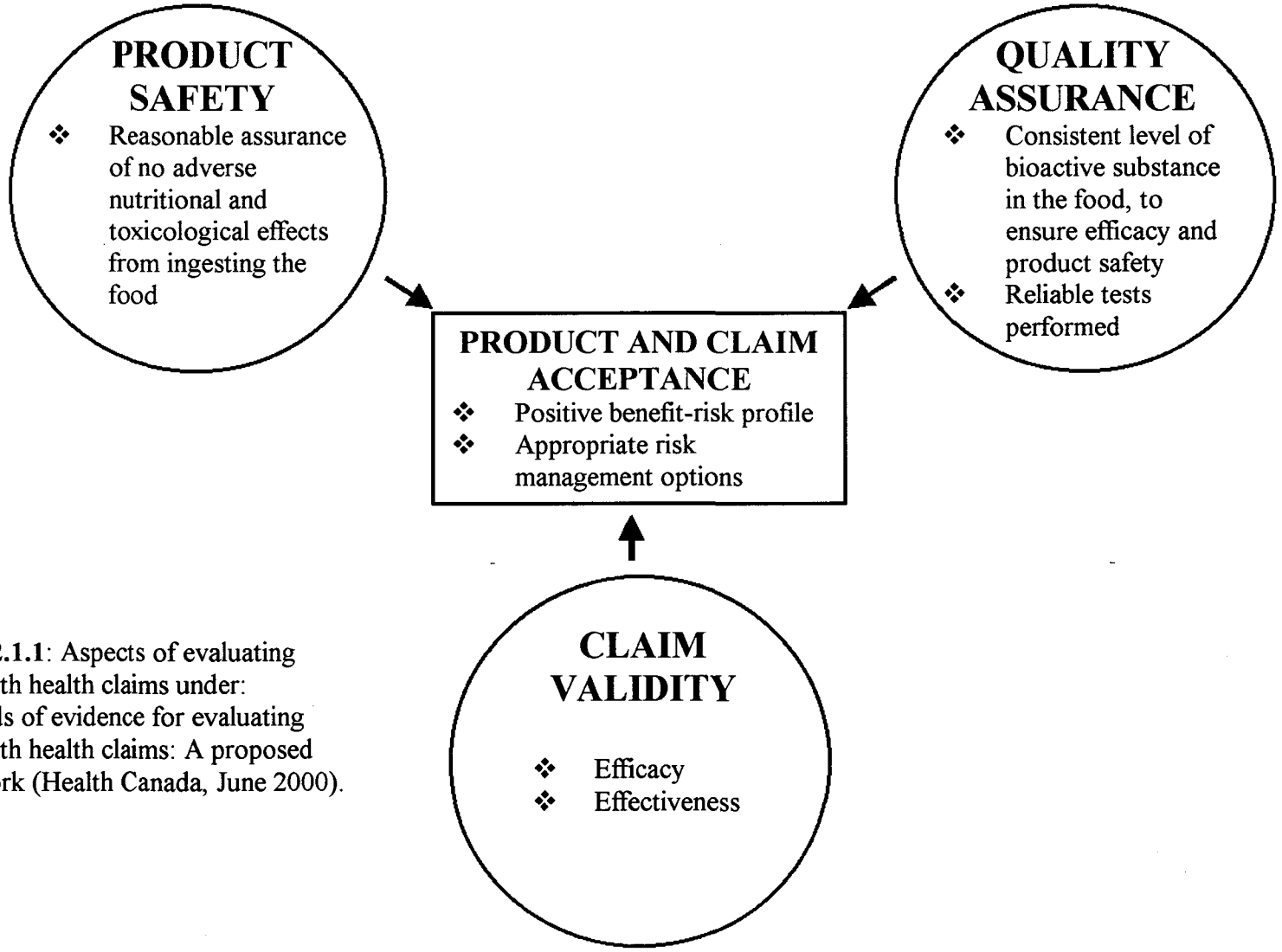


**Figure 2.1.1**: Aspects of evaluating foods with health claims under: Standards of evidence for evaluating foods with health claims: A proposed framework (Health Canada, June 2000).

**Table 2.1.2:** Information required for health claim evaluation [1]

| Safety evaluation | Claim validity evaluation | Quality assurance evaluation |
|---|---|---|
| • Product composition and source, effects of processing, directions for preparation, modification from traditional product<br>• History of safe use/previous human exposure, epidemiological data<br>• Proposed target groups<br>• Dietary significance and physiological role<br>• Identification of susceptible groups<br>• Potential interaction with nutrients/food components<br>• Current exposure and dietary recommendation, upper safety limits, anticipated use, total exposure from different sources<br>• Metabolic fate, metabolic disposition, physiological role<br>• Safety assessment of isolated substance and food matrix | • Experimental and observational studies in humans with acceptable biomarkers supporting the causality of health benefit to food:<br>- Essential criteria:<br>➢ Consistency of findings<br>➢ Magnitude of effect/strength of association<br>➢ Statistical relationship<br>➢ Temporal relationship<br>➢ Absence of strong opposing evidence<br>- Supporting criteria:<br>➢ Dose-response relationship<br>➢ Reversal or cessation of effects<br>➢ Biological plausibility<br>➢ Alternative explanations<br>➢ Specificity of effect and of cause<br>➢ Coherence<br>• Product efficacy and effectiveness<br>• Magnitude of beneficial effect or risk reduction<br>• Sustainability of effect<br>• Recommended intake to achieve effect<br>• Target population and impact on population health<br>• Biological mechanism<br>• Time needed to see an effect | • Critical control points<br>• Specifications and analysis plan<br>• Record retention policy<br>• Recall capability<br>• Evidence of good manufacturing practices<br>• Evidence of good practices in testing procedures<br>• Documentation |

[1] Adapted from Standards of evidence for evaluating foods with health claims: A proposed framework (Health Canada, June 2000).

claim evaluation framework, a basic evaluation of nutritional and toxicological impacts would be conducted for all products bearing health claims, in the context of intended form and intended use. Anticipated exposure to a food, as well as to a bioactive substance, from all sources would then be evaluated, since adverse effects or nutritional imbalance may result at excessively high levels of intake of a health promoting substance. Health benefits of functional foods are likely to be achieved through the addition of bioactive substances at levels higher than those naturally occurring in or currently consumed from the food supply, or the addition of new bioactive substances not traditionally found in foods. Many of these foods would likely meet the criteria for novel foods as defined in the Food and Drug Regulations (schedule 948, Canada Gazette II, 1999). The safety aspects of these foods would have to be assessed before being considered for health claims. In general, safety assessment of foods with health claims would be specific to the novelty and uncertainty regarding the safety of the product, and as such, different levels of scientific evidence and information would be required according to the nature of the food under consideration (Health Canada, June 2000). Post market surveillance may be required to ensure long-term safety in some cases.

#### *2.1.4.2. Claim Validity*

The proposed framework defines guidelines for evaluating claim validity, including concepts of product efficacy and effectiveness. Efficacy entails the demonstration of a beneficial effect under ideally controlled conditions, such as a clinical trial. Effectiveness requires that this effect be observed under average uncontrolled conditions among the population at large. An evidence-based approach, dictated by several principles listed in **Table 2.1.3**, would be used to evaluate claim validity in three

**Table 2.1.3:** Principles of Health Canada's proposed evidence-based approach for evaluating health claim validity [1]

| Validity Principles |
|---|
| • Totality of evidence<br>• Causal relationship<br>• Study quality<br>• Relevance and generalizability<br>• Systematic approach<br>• Level of certainty |

[1] Obtained from Standards of evidence for evaluating foods with health claims: A proposed framework (Health Canada, June 2000).

distinct steps. First, the strength of evidence supporting a causal relationship between a food/bioactive substance and a claimed benefit would be evaluated using a systematic approach, by which the totality of scientific literature is reviewed and conclusions are drawn from the best quality of available evidence. Second, whether the strength of evidence for this causal relationship is sufficient to support the claim, in relation to the nature of the claim, would be determined. Third, whether the total evidence provides sufficient information to characterize the relationship between the claimed benefit and the agent would be established. Characterization encompasses efficacy and effectiveness, level of intake, target populations, and magnitude and sustainability of effect. In addition, the ability to draw conclusions as to the health benefits of functional foods depends on the establishment and validation of suitable biomarkers of surrogate disease endpoints and food intake. The type and extent of evidence required to evaluate foods with health claims would be dependant on the type of claim (i.e. generic/product-specific, structure function/risk reduction), nature and form of food/nutrient, and estimated total daily intake. These guidelines aim to ensure approval of legitimate and substantiated health claims promoted as part of the total diet.

#### *2.1.4.3. Quality Assurance*

Quality consistency of food products with health claims must be maintained if the alleged benefits are to be seen on a population basis. Excessive levels of a beneficial substance and presence of deleterious ingredients must also be prevented to avoid possible health hazards. The level and quality of bioactive substances must be reliably monitored through testing to ensure efficacy and safety of functional foods. The proposed framework defines guidelines on several aspects of quality assurance, namely

good manufacturing practices, good laboratory practices, good practices concerning the collection and analysis of human data, and evaluation of the quality and appropriateness of documentation submitted by applicant companies. These quality assurance requirements of health claim evaluation aim at protecting consumers from fraudulent products with excessive or insufficient levels, or inactive forms, of bioactive substances, as well as from improper testing and data handling practices.

### 2.1.5. Consumer Confidence in Health Claims

Consumer confidence in health claims and the regulatory process through which they are generated must be maintained if effective changes to the nutritional behaviours of Canadians are to be seen. The high standards of evidence elaborated in the proposed framework are directed at protecting health in the general population. The establishment of these stringent guidelines for evaluating product safety, claim validity, and quality assurance should prevent toxicological hazards as a consequence of health claims. Such precautions are essential to maintain consumer confidence in the claims.

Since behavioural changes are complex and difficult to bring about on a population basis, emphasis should be placed on claims having major health impacts. Claims having little or no effect on health should not be allowed, as they may dilute consumers' efforts to modify their eating behavior, and disrupt confidence in the regulatory process and the claims themselves. Unnevehr et al (1998), in a review on health claim regulation in the United States and its impact on consumer choice and protection, suggested the replacement of the gold standard scientific consensus for evaluating claim validity, by a cost-benefit standard of acceptance. The authors

considered that permitting a potentially false but safe health claim would not negatively impact consumers, and therefore, a lower level of scientific substantiation could be required in that case. However, this position underestimates the negative impact that false claims may have on nutritional balance, behavioural changes towards a healthier diet, and health claim credibility. In addition, the consumption of government-authorized health-fraudulent products raises ethical issues and consumer right concerns. It should be considered imperative for consumer confidence in health claims that only credible, legitimate and well-substantiated claims based on established scientific evidence be approved, in order to prevent misleading or deceiving consumers, although extensive scientific evaluation is expected to delay regulatory approval.

Nonetheless, the framework does not clearly define how the difference in criteria stringency required for evaluation of different health claims would be established. How will evaluators deal with missing evidence for one aspect of the safety evaluation when a claim is likely to provide significant health benefits to a large part of the population? Should publication bias and bias of industry-derived research be taken into consideration when evaluating health claim validity? The absence of widely accepted methodologies for measuring biomarkers in certain fields of nutrition, and the different standards for diagnosis establishment in different countries, may also complicate the accurate assessment of claim validity. Moreover, the concept of effectiveness of a health benefit under the population at large may be incompatible with most existing evidence from experimental or observational studies, which are either overly controlled, or confounded by other dietary and environmental factors. It may be appropriate to omit effectiveness from the claim evaluation process but to include it as a measure of claim impact on dietary patterns and health outcomes. Claim validity evaluation may be focused on

efficacy, a reasonable estimation of effectiveness that is obtainable from the scientific literature. Health Canada requires that "the validity of a claim is not likely to be reversed by new and evolving science" (Health Canada, June 2000). This level of certainty may not be realistic, given the nature of scientific research and the likelihood of finding contradictory results. Therefore, it would seem important to include an option for dealing with health claim removal in case of changing evidence in the proposed regulation.

### 2.1.6. Sustainable Development of the Functional Food Industry

Since food industries will be providers of functional foods to the Canadian market, the success of health claim implementation will be dependent on companies' interest to market new products with functional qualities, and to include health claims on existing and future products, a process that will be instigated on a voluntarily basis. Therefore, regulatory impact on functional food industry sustainability will likely affect the availability of foods with health claims, and thus the possibility of consumers to derive health benefits from them. As such, regulators must ensure that generic and product-specific health claim approval is reasonably straightforward and efficient, in terms of approval time, required documents and criteria stringency, to avoid unnecessary burden to food companies, and to ensure a viable future for health claims and functional foods.

While approval of product-specific claims may require extensive testing and documentation, generic claims may be easily applied to a whole range of products once the safety and validity of the biological agent involved has been established. Subsequently, only quality assurance assessments of manufacturing and testing practices,

and perhaps issues of safety and efficacy in different food matrices, would need to be evaluated for products bearing generic health claims. The generic claims currently under evaluation (Table 2.1.1) follow government initiatives, and thus alleviate burden on food companies, as compared to the petition process for health claim approval in use in the United States.

Conversely, the evaluation of product-specific claims requires extensive documentation and scientific literature to describe the different aspects of safety, validity and quality assurance evaluation detailed in Table 2.1.2. The extent to which food companies would be responsible for this extensive documentation has not been established so far in the proposed framework. However, it is certain that the process for submitting health claim proposals must be simplified and clarified to promote the sustainable development of the functional food industry. Furthermore, many aspects of scientific evidence and safety assessment used for claim approval may not be available due to insufficient human studies. Considering the potential health benefits for the population and the costly nature of properly controlled clinical trials in humans, government sources of funding directed to academia may be necessary to promote good quality, unbiased nutrition research.

Regulatory approval for the use of product-specific claims may promote the development of new functional food products and the demonstration of their safe and efficacious nature. Consequently, food industries should be able to derive benefits from their investments in innovative products, and ensure that competitors do not rapidly saturate the market to compromise their margin of profit. However, claims of exclusivity have not been addressed so far within the regulatory framework. The ease with which a competitor could demonstrate that his product is similar in composition and action to an

established one bearing a product-specific claim, without undergoing extensive testing in clinical settings, will likely determine the profitability of developing novel functional foods. Therefore, intellectual property assignment must be made possible, but clearly delimited, as part of health claim regulation in Canada. Since it is inappropriate to restrict diet or food group claims to specific products, patent issues would only be applied to product-specific claims. Restricting claim use of a specific product for a certain time period is expected to promote the development of functional foods with added health benefit, but also to increase prices of these foods due to lack of commercial competition, thus making them only available to a sub-section of the population. Determination of a suitable time period to allow patents on functional foods, which logically should be shorter than that permitted on drugs, may help to keep a balance between these two opposing factors and maximize health benefits to consumers. Such issues have not been addressed so far, and are beyond the scope of the proposed framework for evaluating foods with health claims, but should be addressed in future product-specific regulations.

Positive perception of health claims and functional food products by consumers is critical to the commercial success of these products. Health Canada promotes consumer confidence by ensuring that only safe products bearing legitimate claims and developed under high standards of quality reach the market. The Canadian Food Inspection Agency (CFIA) would be responsible for enforcing compliance of food products to regulatory amendments (Canada Gazette Directorate, 2001). Food companies will need to find strategies to market and advertise functional foods with considerations to product credibility and consumer confidence. Promotion focusing on consumer education to dietary and behavioural practices affecting a particular health outcome will likely be more

successful in ensuring the credibility of the information, as opposed to standard persuasive and sensational marketing strategies.

Development and implementation of health claim regulations can be a lengthy process; however, delaying the introduction of food products bearing health claims on the Canadian market, in an extensive or unreasonable manner, may undermine the commercial and health-benefit success of functional foods. In 1997, the FDA authorised health claims describing the relationship between soluble fibre from whole oats and coronary heart disease, following a petition submitted by General Mills in 1995 (Food and Drug Administration, 1997). While Health Canada's policy regarding health claims was initiated in 1996, it is questionable whether we could expect to see foods with health claims in the local supermarket in a near future. This delay period may promote shifting of interest of consumers or discrediting of functional foods by available but unregulated products, thus preventing the establishment of a flourishing industry and of health benefits to consumers. The recent controversy over the introduction of phytosterol-enriched margarine Becel$^{TM}$ Pro.activ$^{TM}$, for use in reducing blood cholesterol levels, provides an example of possible hazard to the credibility of functional foods and the need for CFIA enforcement. Following introduction of the phytosterol product on the market without official governmental approval, Health Canada issued an advisory (Health Canada, 2001) against plant sterol use in pregnant women, children, people predisposed to hemorrhagic strokes, and in cholesterol-lowering medication users. In response, the manufacturer, Unilever Canada, agreed to include warnings of nutritional inadequacy for those susceptible groups on labels, but continues to stand behind the safety and positive cardiovascular influence of its product (Unilever Canada, 2001). In the case of this functional food, independent experts worldwide and regulatory agencies in Europe, the

United States and Australia have recognized the overwhelming evidence for phytosterols' cholesterol-lowering efficacy (Miettinen et al, 1995; Jones et al, 1999), as well as safety under a large range of experimental conditions, including replicated toxicity assessments (Miettinen et al, 1995; Waalkens-Berendsen et al, 1999; Whittaker et al, 1999). However, these products must be properly evaluated by Health Canada before being available for consumption by the general population, as safety, composition or susceptible group issues may need to be addressed. Time will tell whether this unapproved introduction of Becel™ Pro.activ™ will affect consumers' perception and future use of phytosterol-enriched products. Health Canada is taking the necessary steps toward the eventual establishment of a fully operative administrative body regulating functional foods with the recent publication of its regulatory proposal (Canada Gazette Directorate, 2001). Government action to allow both generic and product-specific claims on functional foods would be desirable within the next 4 or 5 years, given the anticipated health benefits.

### 2.1.7. Effective Communication of Health Claims

Achievement of behavioural changes in the eating habits of Canadians necessitates that health claims be clear, understandable, credible and properly directed to the specific target audience. In addition, the length and amount of information contained in health claims, as well as the association of disease/risk relationships with the food are important. In considering these aspects of effective communication with the consumer, Health Canada has recently conducted a series of focus testing (Golfarb, 2000) in various cities across Canada on the wording of claims, to see how well consumers understand and relate to them. Results of this testing have yielded a number of salient findings regarding

consumer perception of claims. A general lack of basic nutrition knowledge necessary to understand the meaning of claims was found to impact on the perceived value of the claim. Participants stated the need for information on the appropriate intake of a nutrient required to achieve a specific health benefit, based on age, gender, current medical status and family history, as opposed to general information pertaining to the average Canadian. It may be worthwhile, in that respect, to include sensitive groups in the wording of the claims so as to better target populations at risk of a disease/condition and to prevent misuse of a claim by unconcerned populations. For example, the following FDA health claim: "*Regular exercise and a healthy diet with enough calcium help teens and young adult white and Asian women maintain good bone health and may reduce their risk of osteoporosis later in life. Adequate calcium intake is important, but daily intakes above about 2,000 mg are not likely to provide any additional benefit*" (Food and Drug Administration, 1999) provides information on target groups for osteoporosis; however, the rationale for not promoting calcium intake in other subgroups may be questionable. Overall, nutrition education seems necessary to complement the establishment of health claims, in order to help consumers to understand and use health claims in an efficient manner.

Moreover, participants voiced a strong desire for guarantees, and as such, the use of the indefinite concepts "may" or "might" to describe the health benefits of a food or nutrient raised concerns over the usefulness of such a claim; the government was perceived as insufficiently confident in the anticipated benefit. To address this issue, the constantly evolving nature of science and the multi-factorial nature of diseases addressed by health claims should be explained to consumers to justify the necessary use of indefinite terms in health claims. Participants also felt that high standards of evidence

should be required to substantiate claims made, and that supporting evidence should be made available to the public. Use of the name "Health Canada" on claims was considered to promote credibility. It would also be important to investigate whether including product brand names in product-specific claims would increase credibility of the product or be perceived as a promotional tool by consumers.

Some concerns were voiced on the possibility that products containing a beneficial biological agent, but also other harmful agents, be promoted by the use of claims. Significant concerns were expressed with regard to the presence of pesticides and genetically modified products, and the resulting influence of these on health claim validity, especially in the case of fruit and vegetable consumption and cancer risk reduction. Participants in focus groups expressed a desire for claims to be used as educational tools and not just as promotional means for food companies. Thus, it seems that specific requirements for products to bear health claims, as well as tight regulation in the advertisement of claims, are warranted.

The overall objective of health claims to promote a healthier population and reduce strain on the health care system was well understood by participants and was seen as a positive initiative. Testing the specific wording of 4 claims revealed a need for clarification and inclusion of additional information, in order to effectively communicate health messages to consumers. The establishment of a surveillance program that would assess consumer knowledge and attitudes of health claims may be useful in determining whether nutrition messages reach target groups. In addition, the surveillance program would need to monitor changes in food consumption patterns and nutritional status, in order to identify problematic health issues. Such information would be useful in fine-

tuning the regulation system and in developing new and successful products with added health benefits.

### 2.1.8. Case Resolution

After considering the possible benefits that the new calcium-enriched orange juice may have on her family's health, the woman at the grocery store decided to buy the new product. The health claim was therefore effective in communicating the role of calcium to help prevent osteoporosis and to maintain healthy bones, and in reaching the target population, women at risk of osteoporosis. On a national basis, such behavioural changes in eating habits may translate into substantial improvement to the well-being of Canadians and lower health care costs. The actual impact that health claims will have in reducing the risk of chronic diseases over the long run remains to be determined, but it will likely be dependant on a number of other factors, such as the total diet. In addition, physicians and health care professionals are likely to play a crucial role in communicating the usefulness of foods with health benefits to help prevent risk-modifiable diseases to susceptible individuals.

### 2.1.9. Conclusion

Health Canada's proposed framework for evaluating foods with health claims requires high standards of evidence for assessing safety, validity and quality assurance. These requirements should ensure that only safe, legitimate, effective and truthful health claims are used on food products in Canada, therefore maintaining consumer confidence in these claims. The stringency of criteria for evaluating product-specific health claims,

the extent to which companies would be required to provide necessary documentation, as well as the possibility for patent assignment under a future regulatory body, will influence the sustainability of the functional food industry, and thus the availability of foods with added health benefits to consumers. Government initiatives to implement generic health claims are considered to promote claim use by food companies through reduction in their administrative burden, as compared to product-specific claims. Results from focus testing on consumers' perception of health claims revealed that these must be carefully worded, and that consumers must be adequately educated, for health claims to have the anticipated impact on the health of the Canadian population. Health claim regulation should work effectively on these different aspects of a successful claim implementation to ensure that Canadians are provided with a greater variety of safe and efficacious products in the functional food market.

## 2.2. A Functional Oil for the Prevention of Obesity and Cardiovascular Disease

The second part of this literature review examines the evidence for developing a FctO composed of MCT, phytosterols and n-3 PUFA, with possible benefits to weight management and cardiovascular risk profile.

### 2.2.1. Medium Chain Triacylglycerols

Absorption and metabolism of MCFA differ from that of LCFA (**Figure 2.2.1**). During digestion, MCFA are rapidly and completely hydrolysed from the glyceride moiety, since their low molecular weight facilitates the action of pancreatic lipase (Bach and Babayan, 1982). Medium chain fatty acids are then rapidly absorbed from the intestinal lumen into the bloodstream and transported directly to the liver via the portal circulation, thus avoiding storage in peripheral tissues. In contrast, LCFA are packaged into chylomicrons, absorbed through the lymph system, and partly taken up by peripheral tissues before reaching the liver for storage and catabolism (Papamandjaris et al, 1998). The major metabolic route taken by MCFA after uptake by the liver is breakdown to acetyl-CoA, which can be used for lipogenesis, ketogenesis, or alternatively, enter the Krebs cycle for oxidation.

#### *2.2.1.1. Energy Metabolism Modulation*

Medium chain fatty acids are retained in the liver and undergo β-oxidation to a greater extent than LCFA (Johnson et al, 1990), which are minimally oxidized under fed conditions. Reported increases in energy expenditure following MCT ingestion in humans (Seaton et al, 1986; Hill et al, 1989; Scalfi et al, 1991; Mascioli et al, 1991;

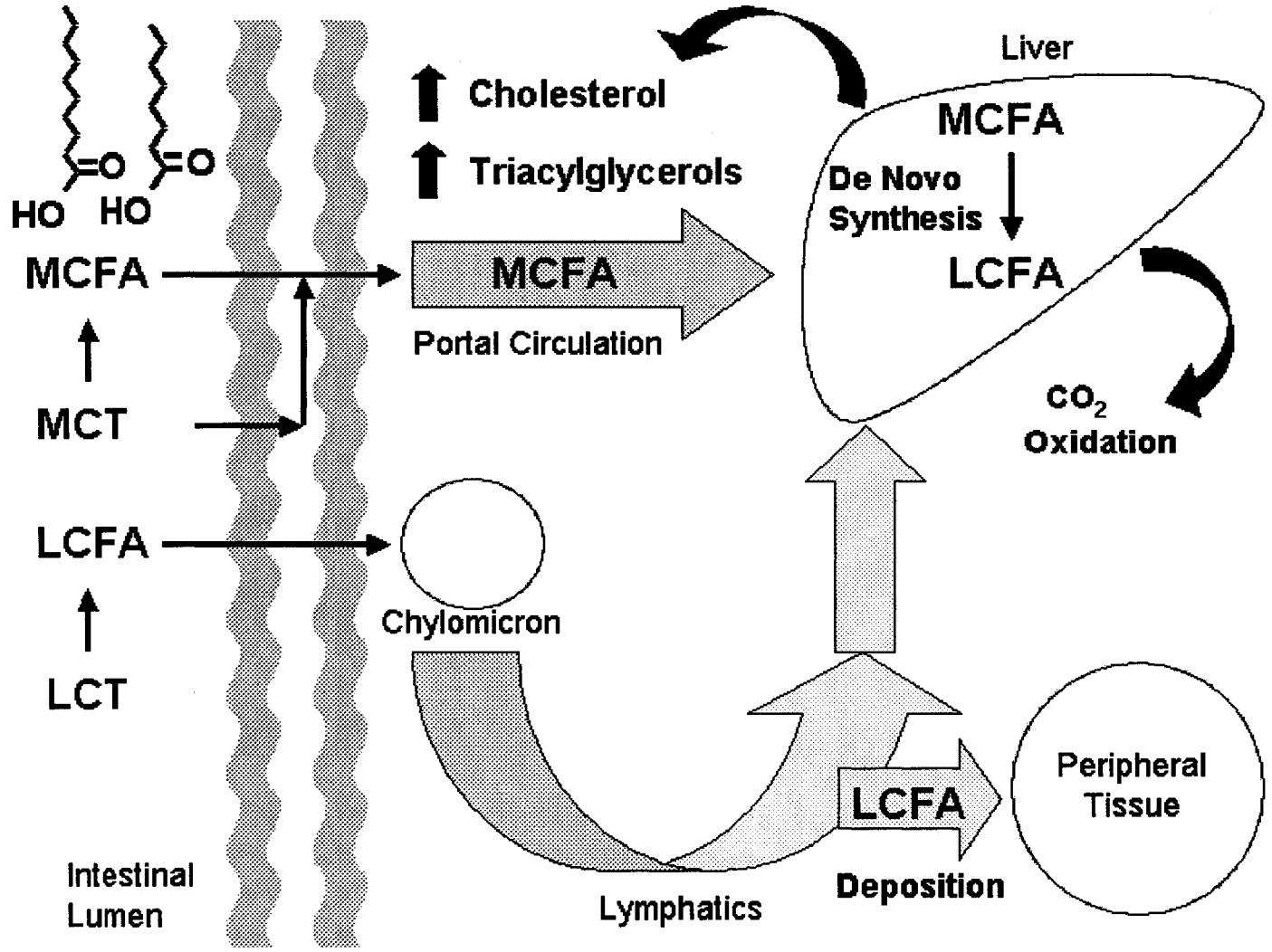


**Figure 2.2.1**: Medium chain triacylglycerols promote negative energy balance through decreased fat deposition, increased fat oxidation and de novo long chain synthesis, but also increase plasma cholesterol and triacylglycerols levels.

Duloo et al, 1996; White et al, 1999; Matsuo et al, 2001) have been attributed to oxidation of MCT to carbon dioxide and de novo synthesis of LCFA. Isotopic measurement of expired $CO_2$ in rats following ingestion of labelled MCT and LCT revealed that the former was oxidized more rapidly and completely than the latter (Johnson et al, 1990). Evidence for de novo FA synthesis from acetyl-CoA subunits, and esterification of those FA into TAG (Crozier, 1988), also confirms the lesser efficiency of MCT for storage. The extra energy invested in de novo synthesis of LCFA from MCT breakdown products reduces the proportion of dietary fat available for storage.

In humans, increased energy expenditure and fat oxidation have been observed following MCT feeding (Seaton et al, 1986; Hill et al, 1989; Scalfi et al, 1991; Mascioli et al, 1991; Duloo et al, 1996; White et al, 1999; Matsuo et al, 2001), as compared to LCT feeding, and these effects were still present after one week of dietary supplementation (Hill et al, 1989; White et al, 1999). A dose response effect of increased thermogenesis with increased MCT consumption has been demonstrated (Dulloo et al, 1996). Whether higher energy expenditure following MCT feeding can be sustained over an extended period of time is still controversial. The enhanced postprandial energy expenditure of MCT was attenuated after 14 days of supplementation (White et al, 1999), suggesting metabolic adaptation to this source of fuel through improved storage efficiency. Adaptation to MCT consumption has also been reported in relation to chylomicron packaging. After 6 days of 8:0 and 10:0 feeding at 40% of energy intake, the presence of those FA in chylomicrons was increased from 8% to 13% (Swift et al, 1990), indicative of adaptation in the route of MCT absorption.

Animal studies indicate that consumption of MCT results in reduced weight gain (Geliebter et al, 1983), as well as in weight loss and reduced fat deposition (Lavau and

Hashim, 1978; Simon et al, 2000) when substituted for equicaloric amounts of LCT. Overfeeding 8:0 and 10:0 in rats reduced weight gain by 20% (Geliebter et al, 1983), while weight-maintenance feeding decreased body weights by 10% (Lavau and Hashim, 1978), when compared to controls fed corn oil. Reductions in adipose tissue of 26 %, and body weights of 10%, were observed after 23 days of MCT feeding in overweight rats, as compared to olive oil feeding (Simon et al, 2000). These studies suggest that MCT may be useful in weight management, due to reduced storage efficiency in adipose tissue.

At present time, the usefulness of long-term MCT supplementation in the prevention of body weight gain and the promotion of weight loss in humans, owing to increased energy expenditure, is not clearly defined. No differential fluctuation in body weights between MCT- and LCT-fed groups was observed after 4 weeks of inpatient, or 12 weeks of outpatient supplementation of hypocaloric diets in obese women (Yost and Eckel, 1989). The same conclusions were reached after 6 weeks of outpatient feeding with MCT and LCT complementing a diet for energy maintenance, in normal-weight and slightly overweight subjects (Temme et al, 1997). Hainer et al (1994) reported that supplementing MCT to a low-calorie diet for 4 weeks provided additional benefits to obesity management, as compared to diet treatment alone. Medium chain triacylglycerols prevented the decrease in resting energy expenditure and plasma HDL cholesterol observed in obese subjects consuming hypocaloric diets alone, although similar weight reductions were achieved in both groups.

Evidence for defective lipid metabolism in obesity has also led to suggest the beneficial replacement of typically consumed LCT by easily metabolized MCT. Binnert et al (1998) observed that obese subjects had a defect in the oxidation of dietary LCT, when compared to lean subjects, and this decreased oxidation was proportional to body

fat mass size. No such alterations in the lipid metabolism of obese subjects were reported when MCT were fed. Thus, replacement of typical fats with MCT may provide benefits to individuals predisposed to obesity due to defective LCT oxidation. Further investigation is needed to confirm the potential long-term benefits of MCT consumption to weight management, through possible increases in energy expenditure, reductions in fat storage efficiency, and bypassing of obesity-related defects in LCT oxidation.

#### *2.2.1.2. Blood Lipid Levels Modification*

Evidence for the effects of MCT consumption on circulating cholesterol and TAG is inconclusive, but unchanged and elevated levels have been reported. In the liver, MCFA are converted to LCFA through de novo synthesis and chain elongation, and resulting LCT are secreted in plasma. Increased levels of palmitic (16:0), stearic (18:0) and oleic (18:1n-9) acids have been observed in circulating TAG following MCT consumption (Hill et al, 1990), thus the presence of these FA likely dictates the cholesterol-modulating effect of MCT.

While lauric acid (12:0) is considered hypercholesterolemic (Katan et al, 1995), reported effects of 8:0 and 10:0, which are potentially greater modulators of energy metabolism, on circulating cholesterol levels are not clearly defined. As early as 1959, Beveridge et al reported a slight rise in plasma cholesterol levels after 16 days of supplementation with MCT oil, which was extracted from coconut oil and contained FA of 6-12 carbons in length. These effects on cholesterol metabolism were of lower amplitude than those of butter and coconut oil. Conversely, 4 weeks of 10:0 feeding as 32% of energy did not change plasma total cholesterol concentrations but increased circulating TAG in chronic schizophrenic male patients (McGandy et al, 1970).

During 6 days of overfeeding inpatient subjects at 150% of energy requirement using a liquid diet composed of 40% fat (61% 8:0 and 32% 10:0), Hill et al (1990) found a threefold increase in TAG levels with MCT, while total and HDL cholesterol levels were unchanged. Soybean oil (32% 18:1n-9 and 51% linoleic acid (18:2n-6, LA)) was the comparison diet and resulted in decreased total cholesterol levels but unchanged fasting TAG. The same laboratory conducted a similar experiment with maintenance energy levels of feeding for 6 days and found a 42% increase in fasting TAG levels, a 15% decrease in HDL cholesterol, and no change in total and LDL cholesterol following consumption of MCT diets of the same composition as the previous study (Swift et al, 1992). This response in blood lipid levels is very similar to that seen with carbohydrates, and supports the view of the neutral nature of MCT on cholesterol concentrations. In addition, lower postprandial triglyceridemia was found with MCT, when compared to LCT.

Wardlaw et al (1995) conducted a study on hypercholesterolemic men consuming the synthetic TAG caprenin, which contains 45% of FA as behenic acid (22:0) and 50% as 8:0 and 10:0, as part of self-selected energy intake diets for 6 weeks. When compared to palm oil/palm kernel oil baseline values, caprenin decreased HDL cholesterol while total and LDL cholesterol, and TAG levels remained stable. Compared to a butter fat baseline diet, caprenin had no effect on lipid levels. Therefore, the TAG caprenin may not be considered beneficial toward cholesterol metabolism, since it was found equally unfavourable as the atherogenic palm oil, palm kernel oil and butter. However, the exact involvement of 8:0 and 10:0 to this effect is not clear.

Cater et al (1997) treated 9 mildly hypercholesterolemic male inpatients with a weight-maintaining diet providing 43% of energy as treatment fat, which consisted of

MCT (68% 8:0 and 32% 10:0), palm oil and sunflower oil, and was administered in a crossover design. Total cholesterol concentrations following MCT and palm oil feeding were not significantly different from one another but were higher in comparison to sunflower oil feeding. LDL cholesterol concentrations paralleled those of total cholesterol while HDL cholesterol levels remained unchanged. Levels of TAG were higher during the MCT diet than either of the palm oil or sunflower oil diets. These results suggest a negative impact of MCT on blood lipid levels.

A trial carried out on 60 outpatient subjects to investigate the effect of dietary FA composition on lipoprotein levels consisted of a 3-week run-in period, followed by a 6-week treatment phase, during which subjects received either MCT (33% 6:0, 25% 8:0, 42% 10:0), myristic acid (14:0), or 18:1n-9 as the 10% treatment fat, with 40% of energy provided by fat (Temme et al, 1995). In this setting, MCT increased TAG slightly and unfavourably influenced the apo A-I/ApoB ratio. Low density lipoprotein cholesterol levels were not affected. However, this design did not allow for reliable measurement of compliance, therefore, it cannot be confirmed whether subjects indeed consumed the foods they were instructed to. In addition, the observed changes in blood lipid parameters in this study may not reflect the amplitude with which MCT consumption modulates cholesterol levels, given the low levels of treatment fat administered.

Tsai et al (1999) studied the effect of MCT feeding on the cholesterol metabolism of 17 premenopausal women, using a crossover design. Subjects were fed a baseline diet composed of 10% of energy coming from PUFA and 10% from saturated fat for one week before each phase. Then, they received 14% of energy, or 30% of fat, as 8:0 and 10:0, or 12:0, for 4 weeks, with a 7-week washout period between phases. Monounsaturated fat and dietary cholesterol were kept constant between baseline, 8:0 and

10:0, and 12:0 diets. Compared to the PUFA baseline diet, MCT had 2/3 the cholesterol-raising potency of 12:0. Plasma LDL and total cholesterol were raised by 11% and 7%, respectively, on the 8:0 and 10:0 diet, and by 16% and 12% on the 12;0 diet. Consumption of MCT had no effect on circulating TAG and HDL cholesterol levels. In contrast to 12:0, 8:0 and 10:0 were found to raise the receptor-mediated degradation of LDL, even though levels of circulating LDL were increased. Perhaps LDL clearance was overcome by the important increase in LDL production.

Through step-wise increments in the proportion of MCT intake relative to corn oil in hypertriglyceridemic subjects, 100% of treatment fat, or approximately 64% of total fat, was provided as MCT after 4 weeks (Asakura et al, 2000). In this population group, MCT resulted in higher fasting total cholesterol relative to corn oil, but TAG concentrations were not different from those of low fat or corn oil-enriched diets.

Since consumption of MCT results in unchanged or slightly raised plasma cholesterol and TAG levels, these fats may represent a less favourable alternative compared to lipid-lowering PUFA. However, combining MCT with cholesterol-lowering phytosterols and TAG-suppressing n-3 PUFA could prevent possible undesirable influences of MCT on cholesterol and TAG metabolism.

### 2.2.2. Phytosterols as Cholesterol-Lowering Agents

Phytosterols are cyclic fat-soluble compounds isolated from plant material. They are structurally similar to cholesterol, with a methyl or ethyl group substitution at the C24 position constituting the small difference in molecular structure between these sterols. Due to this molecular discrimination, phytosterols are minimally absorbed from the

gastrointestinal lumen, while they block the absorption of dietary and endogenously derived cholesterol, through competition for micelle formation or precipitation of cholesterol into an insoluble form (Jones et al, 1997) (**Figure 2.2.2**). Inhibition of cholesterol absorption with consumption of moderate quantities of phytosterols has been shown to significantly lower blood cholesterol levels in both hypercholesterolemic (Lees et al, 1977; Heinemann et al, 1986; Vanhanen et al, 1993; Miettinen et al, 1995) and normocholesterolemic (Pelletier et al, 1995; Hendriks et al, 1999; Plat and Mensink, 2000; Tammi et al, 2000) subjects.

Lees et al (1977) observed a 12 % decrease in the plasma cholesterol levels of hypercholesterolemic subjects, following administration of either saturated or unsaturated phytosterols. Three grams of saturated tall oil stanols per day were found just as efficacious as 18g of unsaturated soy sterols in lowering cholesterol levels.

Consumption of low doses of sitostanol, 1.5g per day, was also observed to reduce cholesterol in hypercholesterolemic patients (Heinemann et al, 1986). A 10% decrease in circulating total cholesterol levels was achieved after 3 weeks of supplementation, and a 15% decrease was observed after 4 weeks. This reduction in total cholesterol was entirely due to a fall in LDL cholesterol, thus achieving a most favourable HDL:LDL ratio.

Miettinen et al (1995) carried a one-year long, randomized, double blind trial on 153 mildly hypercholesterolemic subjects. Consumption of a sitostanol-ester containing margarine, providing 1.8g or 2.6g of sitostanol per day, was found to reduce total and LDL cholesterol concentrations by 10% and 14%, respectively, and additional cholesterol-lowering was found at higher phytosterol dose. No change in TAG or HDL cholesterol levels was observed. Long-term consumption of sitostanol at those levels was

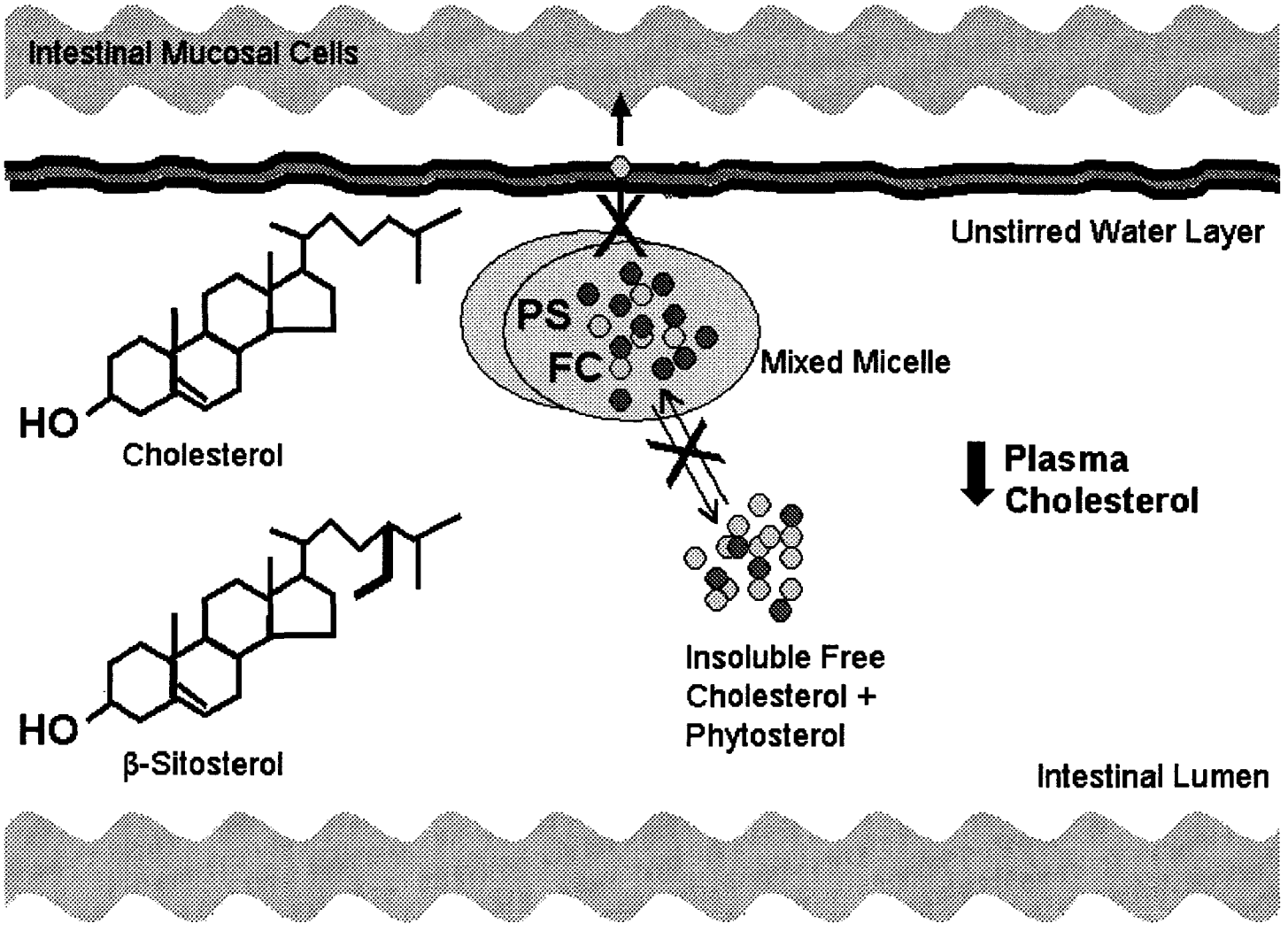


**Figure 2.2.2**: Phytosterols are structurally similar to cholesterol and lower plasma cholesterol levels by blocking intestinal cholesterol absorption, through micelle exclusion or precipitation of cholesterol into an insoluble form.

found safe, devoid of side effects and efficacious in lowering blood cholesterol in hypercholesterolemic subjects. In a parallel study, hyperlipidemic male subjects were fed precisely controlled diets supplemented with either 1.7g of sitostanol per day, or placebo as control (Jones et al, 1999). Phytosterols resulted in lower plasma total cholesterol levels at day 30 in comparison to the control diet, and a time effect was also observed. A significant 24% decrease in LDL cholesterol was observed in the treatment group, vs a 9% decrease in the control group.

In a study by Pelletier et al (1995), phytosterol supplementation was found effective in reducing lipoprotein levels in normocholesterolemic subjects. Only 740mg of soybean phytosterols consumption for 4 weeks lowered total cholesterol by 10% and LDL-cholesterol by 15%, thus increasing the protective HDL:LDL cholesterol ratio by 25%. These results provide evidence that modest intake of phytosterols can lead to improved cardiovascular risk profile in the general population.

Other phytosterol supplementation studies have provided evidence for reduction in atherogenic LDL cholesterol, in a range of 6.7-15%, in individuals with normal cholesterol levels (Weststrate et al, 1998; Hendriks et al, 1999; Plat and Mensink 2000). Moreover, similar cholesterol lowering has been found with saturated and unsaturated sterols (Weststrate and Meijer, 1998), variable fat sources (Gylling and Miettinen, 1999; Jones et al, 2000), and low fat diets (Hallikainen et al, 2000). In addition, hypercholesterolemic individuals on statin therapy were reported to derive additional reductions in cholesterol concentrations following stanol ester consumption (Blair et al, 2000).

The cholesterol-lowering properties of phytosterols may be limited when these agents are incorporated to diets low in cholesterol. In contrast to previous studies, Denke

(1995) failed to detect a change in LDL cholesterol following intakes of relatively large doses (3g/day) of the saturated sitostanol in hypercholesterolemic men, when dietary cholesterol was restricted to < 200mg/day. The ratio of phytosterol to dietary cholesterol may be an important factor influencing plant sterol's ability to lower blood cholesterol, since these compounds may be more efficient at precipitating exogenous vs endogenous sterol compounds (Jones et al, 1997). Pelletier et al (1995) recommended a phytosterol:cholesterol ratio around 2 to achieve optimal cholesterol-lowering. Another possible reason for the absence of cholesterol lowering in the study by Denke (1995) may be related to the vehicle of administration of phytosterols. In fact, capsules were used and these may not have allowed for sufficient contact between phytosterols and dietary cholesterol for the former to effect precipitation and block of absorption of the latter. Incorporation of phytosterols to the fat-soluble fractions of meals may be needed for dispersion and maximal intestinal availability, and is expected to lower both exogenous and endogenous sterol absorption.

Since fat-soluble vitamin and carotenoid absorption is dependent on micelle formation, the effect of phytosterol supplementation on levels of these nutrients has been investigated. Phytosterols were not found to affect plasma levels of lipid standardized vitamins A, D, E or K (Hendriks et al, 1999; Gylling and Miettinen, 1999; Hallikainen et al, 2000). Decreases in standardized beta-carotene concentrations, by 8-30%, have been observed (Hendriks et al, 1999; Gylling and Miettinen, 1999; Tammi et al, 2000), although values remained within normal ranges and reported reductions were comparable in magnitude to that of seasonal variation. Moreover, several toxicity studies have been completed to investigate the safety of phytosterols at very high intake. No detrimental effect of phytosterols has been observed on hormonal, reproductive, developmental or

metabolic indices (Waalkens-Berendsen et al, 1999; Whittaker et al, 1999; Ayesh et al, 1999).

The safe nature of phytosterols has been further demonstrated in children of 6 years of age who participated to a supplementation study, which aimed at reducing exposure of young children to atherosclerosis risk factors (Tammi et al, 2000). Significant reductions in total and LDL cholesterol, by 5.4% and 7.5% respectively, have been observed after 3 months of well-tolerated phytosterol feeding. Plasma HDL cholesterol, TAG and alpha-tocopherol:LDL cholesterol ratio were unchanged, however, standardized beta-carotene was decreased but remained within normal range.

The established efficacy and safety of plant sterols and stanols in reducing plasma cholesterol concentrations provide a rationale for incorporating these sterols to a FctO with antiatherogenic properties. The possible negative effects of MCT on cholesterol metabolism could thus be counteracted by addition of cholesterol-lowering phytosterols.

### 2.2.3. n-3 Polyunsaturated Fatty Acids as Triacylglycerol-Lowering Agents

Consumption of n-3 PUFA has been associated with decreased mortality from CVD (Kromhout et al, 1985). Epidemiological studies indicate that fish eating populations, such as the Greenland Eskimos, have a low prevalence of coronary heart disease and improved blood lipid parameters, compared to Danes (Bang and Dyerberg, 1980). The preventive effect of n-3 PUFA on heart disease has been ascribed to their favourable influence on plasma TAG and VLDL levels (Katan et al, 1995). In addition, n-3 PUFA have been observed to influence a number of physiological processes implicated in CVD, such as prevention of arrhythmias, conversion to prostaglandin and

leukotriene, eicosanoid synthesis, antiinflammation, inhibition of cytokines and mitogen synthesis, stimulation of endothelial-derived nitric oxide, improved platelet function, antithrombogenesis, and inhibition of atherosclerosis (Katan et al, 1995; Harris, 1997; Connor, 2000).

#### *2.2.3.1. Marine n-3 Fatty Acids*

The n-3 PUFA mostly studied in relation to CVD are those derived from fish oils, including EPA and DHA. A meta-analysis by Harris (1997) looking at the effect of n-3 PUFA on blood lipid levels revealed that fish oil FA are potent hypotriacylglycerolemic agents; their substitution for 1% of energy decreases serum TAG levels by as much as 30%. Mechanisms responsible for the observed decline in TAG may involve actions of EPA and DHA to decrease hepatic VLDL production, and to increase the lipoprotein lipase-mediated uptake of TAG-rich lipoproteins by adipose tissues (Roche and Gibney, 2000). Examination of crossover and parallel studies involving marine n-3 PUFA feeding revealed that total cholesterol remains unaffected, LDL cholesterol is increased by 5-10%, and HDL cholesterol is raised by 1-3% following their supplementation (Harris, 1997). Katan et al (1995) also reported increases in LDL cholesterol in several fish oil feeding studies. Thus, marine n-3 PUFA do not seem to share the cholesterol-lowering abilities of n-6 PUFA (Katan et al, 1995; Kris-Etherton and Yu, 1997).

#### *2.2.3.2. Alpha Linolenic Acid*

The plant n-3 PUFA, ALA, is a precursor to EPA and DHA and is present as 50% of FA in flaxseed oil (Cunnane et al, 1993). High-ALA feeding (13.7g/day) in humans has been found to result in a 2.5 fold increase in EPA levels in plasma phospholipids and

neutrophil phospholipids (Mantzioris et al, 1994). Other studies have observed increases in tissue long chain n-3 PUFA following flaxseed (Cunnane et al, 1993) or flaxseed oil (Indu and Ghafoorunissa, 1992; Layne et al, 1996; Fokkema et al, 2000) consumption, although the levels reached were lower than those resulting from fish oil consumption. Stable isotope studies in humans have established that conversion of ALA to the long chain EPA and DHA does occur under habitual intake conditions. Emken et al (1994) reported that the conversion of ALA to EPA was more efficient when the basal diet was low vs high in LA, 8% vs 3.4%, and similarly for conversion to DHA, 4.0% vs 3.6%. Conversely, Pawlosky et al (2001) observed that only about 0.2% of plasma ALA was destined to EPA synthesis. However, the low amount of ALA provided by the basal diet during the 21-day adaptation period, 0.72g/day, may have promoted ALA deficiency in some subjects. Thus, a large portion of administered deuterated ALA may have been utilised for essential cellular functions, at the expense of downstream conversion to long chain n-3 PUFA. In addition, at high-ALA intakes, substrate inhibition seems to reduce conversion of ALA to EPA. Indeed, a surprisingly lower extent of ALA conversion to EPA was observed at high ALA intake (8.3 g/d), in comparison to oleic acid (8.3 g/d) (Vermunt et al, 2000).

Thus, evidence for ALA conversion to long chain n-3 PUFA in vivo provides a rationale for TAG-lowering following ALA ingestion (**Figure 2.2.3**). In fact, Singer et al (1986) observed decreased TAG levels by 22-24% following 2 weeks of ALA feeding at a dose of 38mL (20-25g) per day, when compared to LA feeding. However, no change in TAG was observed in a subsequent study by Layne et al (1996), where a lower dose of ALA (2.5g/day) was supplemented for 3 months, and compared to olive and fish oil feeding. Abbey et al (1990) reported TAG lowering with fish oil supplementation, but no

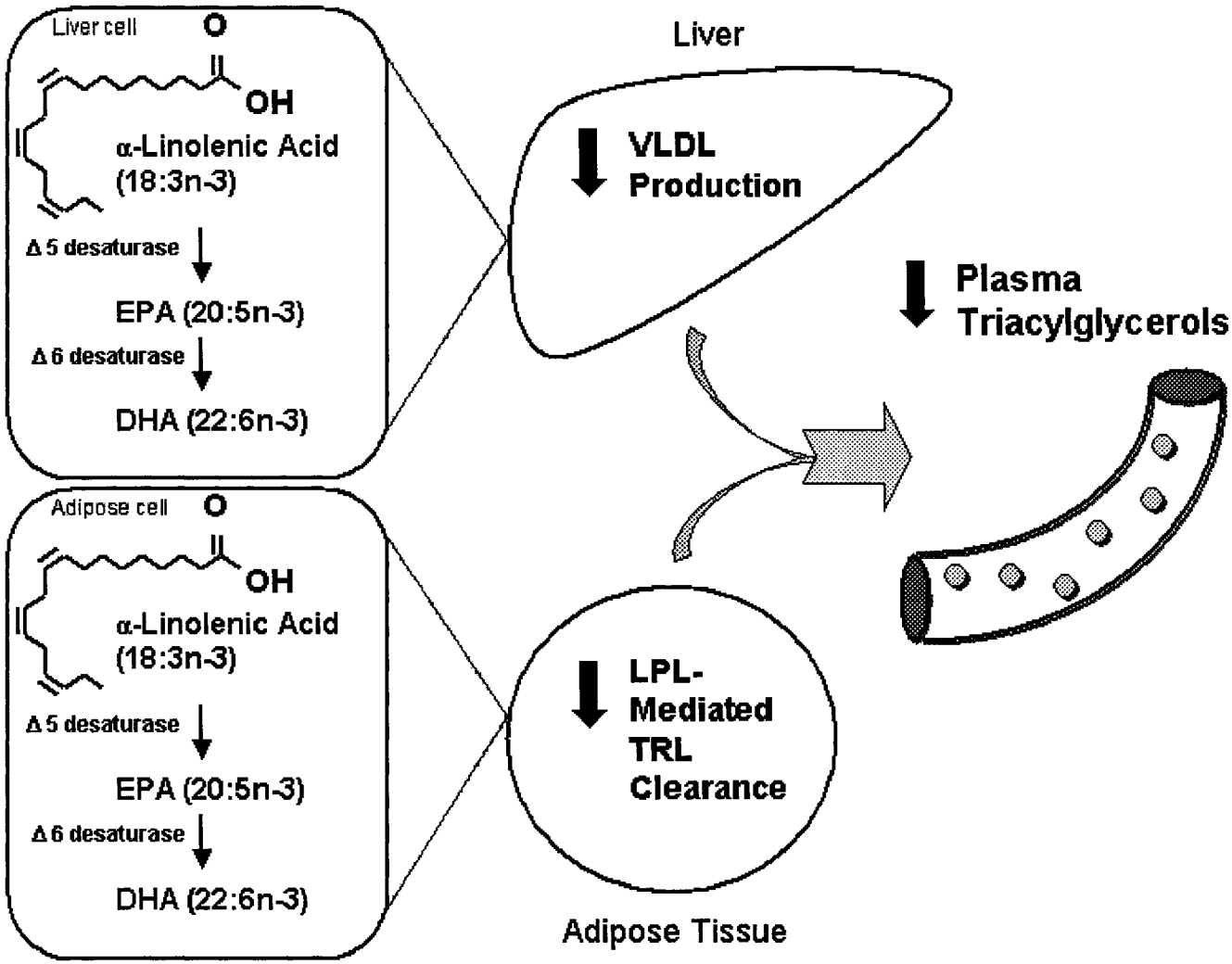


**Figure 2.2.3**: Conversion of alpha-linolenic acid to the long chain n-3 fatty acids eicosapentaenoic and docosahexaenoic acids provides a rationale for lowering plasma triacylglycerol levels, through potential decrease in hepatic very low density lipoprotein secretion and increase in lipoprotein lipase-mediated uptake of triacylglycerol-rich lipoproteins by adipose tissue.

change was observed with flaxseed or safflower oil feeding. Even when using a 2-week stabilization period with controlled diets, supplementation of ALA did not affect blood lipid levels differently than LA feeding (Pang et al, 1998). Flaxseed oil feeding at 2 different ratios of polyunsaturated to saturated FA also failed to reduce fasting TAG levels in non-insulin-dependent diabetic subjects (Goh et al, 1997). Conversely, with background diets low in total and saturated fat, and while keeping LA constant, 3.7g of ALA were found similarly efficacious as 0.3g of long chain n-3 PUFA in lowering TAG concentrations (Indu and Ghafoorunissa, 1992). A LA:ALA ratio of 4 was used in this study and was considered to favour conversion to long chain fats and lowering of TAG levels.

The small number of well-controlled studies looking at blood lipid modification following flaxseed oil supplementation may not allow for definite conclusions as to ALA action on cholesterol levels. Analysis of available studies (Harris, 1997) revealed that, in contrast to marine n-3 PUFA, ALA produced changes in lipoprotein levels that were not different from those of n-6 PUFA, thus favourably influencing cholesterol metabolism. Furthermore, four weeks of flaxseed consumption (50g/day) decreased total cholesterol levels by 9% and LDL cholesterol levels by 18%, although it is not clear whether the mucilage in the flaxseed, ALA, or simply the displacement of saturated fat intake was responsible for this cholesterol-lowering effect (Cunnane et al, 1993). In a study by Chan et al (1991), controlled diets were supplemented for 18 days with four oils/oil mixtures (26% of energy): sunflower and olive; canola; soybean; and sunflower, olive, and flax oils. These oil combinations were found to be equally hypocholesterolemic, and thus it was concluded that ALA, LA and oleic acids were as effective in lowering blood cholesterol levels, when substituted for saturated fat. Additional comparisons of flaxseed

oil with other fat sources in controlled dietary interventions may be needed to further define the relative cholesterol-modulating ability of ALA. The established role of marine n-3 PUFA as TAG-lowering agents, and the conversion of ALA to EPA and DHA in vivo, suggest that the incorporation of flaxseed oil into the present FctO may promote maintenance of healthy plasma TAG concentrations by counteracting the possible TAG-raising effect of MCT.

### 2.2.4. Homocysteine: A Cardiovascular Risk Marker

Homocysteine is a breakdown product of amino acid metabolism, and has been recognized as an independent risk marker for the development of CVD (Stampfer et al, 1992; Nygard et al, 1995). Folate, vitamin $B_6$ and vitamin $B_{12}$ are essential for proper metabolism of Hcy, and to prevent its accumulation. Reasons for abnormally elevated levels of Hcy include: genetic defect in the enzymes involved in amino acid metabolism, vitamin deficiency, renal failure and other chronic conditions (Hankley and Eikelboom, 1999). However, moderate elevations in Hcy concentrations seen in CVD patients are of physiological importance and may not be solely attributed to vitamin deficiencies.

The mechanism by which Hcy may cause vascular damage has not been elucidated so far. Evidence suggests that Hcy may promote atherogenesis and thromboembolism by increasing arterial injury and lipid peroxidation directly, or by producing oxidation products such as hydrogen peroxide and superoxide anion radicals. Homocysteine may also damage the vascular matrix, and promote proliferation of vascular smooth muscle cells. In addition, possible consequences of high Hcy levels are alteration of surface endothelial cell properties to a procoagulant phenotype that may

result in endothelial-leukocyte interactions, impairment to the endothelium vasomotor regulation, and platelet activation (Jacobsen, 1998; Hankley and Eikelboom, 1999; Thambyrajah and Townend, 2000). Potential mechanisms for Hcy promotion of heart disease are summarized in **Figure 2.2.4.**

#### *2.2.4.1. Relationship Between Homocysteine and Cholesterol*

Levels of Hcy and cholesterol, both risk markers for CVD, have been correlated in numerous studies. In the Hordaland Hcy study (Nygard et al, 1995), carried in Norway on more than 16,000 participants, Hcy was correlated with several independent risk factors for CVD, such as smoking, male gender, old age, hypertension, heart rate, sedentary lifestyle and cholesterol levels. A strong positive correlation between total cholesterol and Hcy was observed, even after adjustment for vitamin supplements, and fruit and vegetable consumption.

In a study by Olszewski et al (1989) on 52 men, half of which had coronary heart disease and the other half were free of disease, levels of Hcy were correlated with plasma total cholesterol, fasting TAG and body mass index. Furthermore, supplementation of a subgroup of heart disease patients with pyridoxine, folate, cobalamin, choline, riboflavin and troxerutin for 21 days lowered Hcy by 68%, and also reduced total cholesterol by 79%, while HDL cholesterol remained unchanged.

Olszewski and McCully (1991) looked at the Hcy content in different lipoprotein fractions in hyper- vs normocholesterolemic subjects, and found higher Hcy in the LDL, VLDL and HDL fractions of hyperlipidemic men, when compared to controls.

McCully et al (1990) used a rabbit model to look at the effect of feeding a synthetic atherogenic diet, and of exposure to highly reactive Hcy derivatives, on the

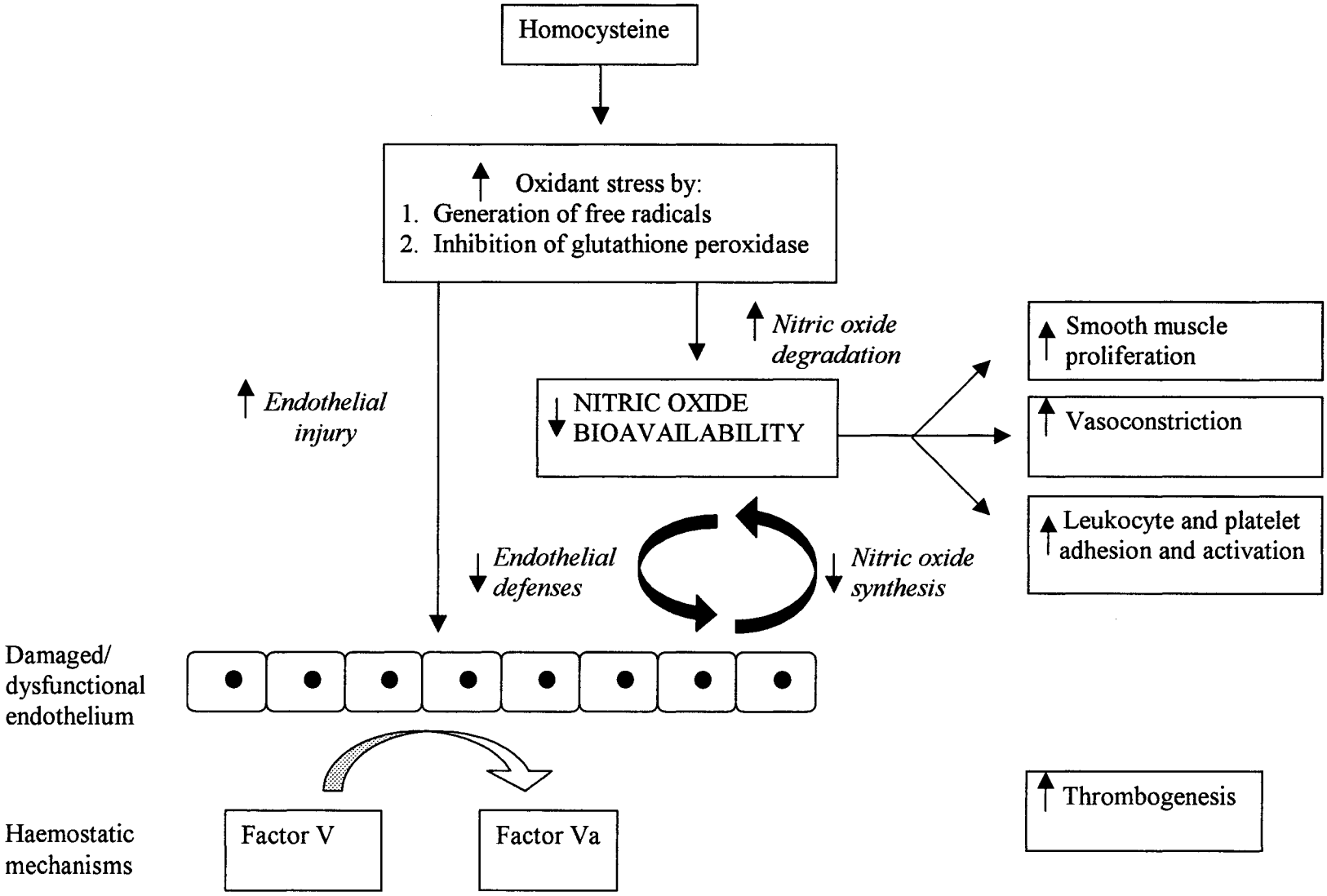


**Figure 2.2.4**: Potential atherogenic mechanisms of homocysteine. (Obtained from Thambyrajah and Townend, 2000)

extent of atherogenic lesions. Aortic atherosclerotic plaques were found to be correlated with serum concentrations of total Hcy (r=0.54, $P<0.001$), total cholesterol (r=0.46, $P<0.05$) and LDL + VLDL cholesterol (r=0.43, $P<0.01$). Moreover, serum levels of Hcy were associated with total cholesterol (r=0.68, $P<0.001$), LDL + VLDL cholesterol (r=0.61, $P<0.001$) and HDL cholesterol (r=0.66, $P<0.001$). This study provides evidence that Hcy facilitates atherogenesis when given along with a diet high in saturated fat and cholesterol. A subsequent study in rats reported a synergy between Hcy and cholesterol feeding in raising total plasma Hcy, cholesterol and TAG levels (Zulli et al, 1998).

Even though Hcy and cholesterol seem to be intrinsically related in the genesis of atherosclerosis, the effect of FA, being the most important dietary determinants of blood cholesterol, on Hcy levels are not known to a great extent. Given that folate, vitamins $B_6$ and $B_{12}$ supplementation improve both Hcy and cholesterol levels (Olszewski et al, 1989), it is reasonable to inquire whether attempts to improve cholesterol levels through dietary FA modification may also benefit Hcy levels.

#### *2.2.4.2. Effect of Dietary Fat on Homocysteine*

Two studies have attempted to verify whether the protective attributes of fish oil n-3 PUFA to CVD prevention are mediated through changes in Hcy levels. Olszewski and McCully (1993) used a crossover design to compare fish and olive oil consumption, at a level of 12g per day for 3 weeks, under controlled dietary conditions in hyperlipidemic subjects. Fish oil resulted in a 48±33% decrease in Hcy levels in 7 out of 9 subjects who received fish oil first, and in a 36±22% decrease in 7 out of 8 of those subjects who received olive oil first. Consumption of fish oil also reduced TAG and VLDL cholesterol, but had no effect on total, LDL or HDL cholesterol levels. In trying

to replicate these results, Grundt et al (1999) re-examined blood samples from a previous study in which subjects were given 2g per day of an EPA and DHA mixture in capsule form for 12 weeks. In this trial, no change in Hcy concentrations was found following fish oil consumption when compared to corn oil consumption. Moreover, Hcy was not correlated with TAG, total or HDL cholesterol. It should be noted that in the study by Grundt et al (1999), diets were not controlled for and a parallel design was used, thus introducing variability in the Hcy values. These factors, in addition to the lower dose of fish oil supplemented, could explain the lack of Hcy modulation, as well as the absence of an association between Hcy and cholesterol, observed in this study.

Preliminary evidence suggests that saturated and monounsaturated FA do not influence circulating total Hcy concentrations. Three weeks of olive oil supplementation failed to alter plasma total Hcy in humans (Olszewski and McCully, 1993), as did 24 weeks of butter feeding in rabbits, as compared to chow feeding (McCully et al, 1990). In contrast, corn oil feeding, which contributes n-6 PUFA, was shown to result in lower serum total Hcy levels, as compared to chow feeding in rabbits (McCully et al, 1990). Conversely, Grundt et al (1999) observed no change after corn oil supplementation for 12 weeks in humans, although design, diet and dose considerations mentioned above may have prevented these investigators from effectively assessing the effect of corn oil on plasma total Hcy.

Given that cholesterol metabolism has been shown to influence Hcy metabolism, dietary FA affecting plasma cholesterol levels may also modulate, directly or indirectly, total Hcy levels in circulation. Since PUFA have been suggested to positively influence Hcy levels, their incorporation into the present FctO may further improve the CVD profile by lowering circulating total Hcy levels.

## 3. RATIONALE

Obesity constitutes an increasingly widespread health problem in Western countries and is associated with elevated risk for a number of health-threatening conditions, such as CVD and cancer, which are the two most common causes of mortality in our society. Dietary interventions directed at controlling body weight would thus result in considerable health benefits. Medium chain triacylglycerol feeding increases energy expenditure when compared to feeding of typically consumed long chain fats (Hill et al, 1989; White et al, 1999); thus, long-term supplementation of MCT may promote maintenance of normal body weights, or even weight loss, through a negative influence on energy balance. However, the potential benefits of MCT to weight control may be counteracted by their detrimental influence on blood lipid parameters, and thus on the cardiovascular risk profile. Development of a FctO having potential attributes to increase energy expenditure and reduce plasma lipid levels would permit the use of MCT in weight management and provide maximal health benefits to populations at risk. Incorporation of phytosterols, which have established cholesterol-lowering properties (Miettinen et al, 1995; Jones et al, 1999), to the FctO should counteract MCT-derived increases in plasma cholesterol levels. Marine n-3 PUFA have potent TAG-lowering activity (Harris, 1997), and may also favourably influence Hcy levels (Olszewski and McCully, 1993), a risk factor for CVD. Addition of ALA, a precursor to the long chain n-3 EPA and DHA (Mantzioris et al, 1994; Layne et al, 1996), to this FctO should neutralize reported increases in TAG levels following MCT consumption, and may also decrease Hcy levels. Plasma concentrations of cholesterol, TAG and Hcy following FctO consumption were assessed to ensure cardiovascular safety.

Medium chain fatty acids are converted to long chain fats in the liver, and those have a direct effect on cholesterol metabolism. Therefore, FA composition of red blood cells, a measure of long term FA consumption, and relationship between changes in these tissue FA and plasma cholesterol levels may provide information on the cholesterol-modulating effect and mechanism of MCT. Conversion of ALA to the long chain EPA and DHA is regarded as critical for reduction in circulating TAG concentrations. Presence of these n-3 FA in red cell tissue following supplementation of the FctO may provide information on the extent of ALA conversion under the present experimental conditions, and correlations with changes in TAG may provide insight into the mechanism involved in the potential TAG-lowering effect of ALA. In addition, since MCT are absorbed through the portal vein, they may influence absorption of other FA, which require chylomicron packaging for transport across the intestinal mucosa. Preferential intestinal absorption of FA following feeding with the FctO may influence changes in blood lipid parameters.

Homocysteine is now recognized as a risk factor contributing to the development of CVD and has been associated with other risk factors, such as cholesterol in plasma (Stampfer et al, 1992; Nygard et al, 1995). Vitamin supplementation has lowered both cholesterol and Hcy (Olszewski et al, 1989), while atherogenic diets containing saturated fat and cholesterol increased both Hcy and cholesterol (McCully et al, 1990). A synergy was also observed between dietary Hcy and cholesterol in raising plasma total Hcy, cholesterol and TAG concentrations (Zulli et al, 1998). Given that these two CVD risk factors appear to be linked in the development of atherogenesis, FA dietary interventions directed at modifying cholesterol levels may also influence Hcy metabolism.

# 4. HYPOTHESIS AND OBJECTIVES

## 4.1. Hypothesis

Combining MCT with phytosterols and n-3 PUFA prevents elevations in plasma cholesterol and TAG levels, when consumed by normo- and moderately hypercholesterolemic overweight women under controlled conditions, as compared to beef tallow feeding.

## 4.2. Objectives

### 4.2.1. Main Objective

The objective of this study was to determine the effect of supplementing precisely controlled diets for 27 days in an inpatient setting, with a FctO composed of MCT, phytosterols and n-3 PUFA, and compared with beef tallow as control, on cardiovascular risk of overweight women.

### 4.2.2. Specific Aims

The specific aims of this study were to examine the effect of dietary fat modulation on:

(i) Plasma total, LDL and HDL cholesterol, and TAG concentrations

(ii) Plasma total Hcy and other aminothiol concentrations

(iii) FA composition of red blood cells and fecal samples

(iv) Relationships between changes in plasma lipid concentrations and changes in body weight, red cell FA, and fecal FA excretion

## 5. Manuscript 2

# Consumption of a functional oil composed of medium chain triacylglycerols, phytosterols and n-3 fatty acids improves the overall cardiovascular risk profile of overweight women

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The results of this study have been presented in part at the FASEB conference in Orlando, US (March 31-April 4, 2001), and at the International Congress of Nutrition in Vienna, Austria (August 27-31, 2001).

Running head: MCT, phytosterols, n-3 fats: lipid metabolism

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## 5.1. Abstract

**Background:** Medium chain triacylglycerols (MCT) have been suggested as efficacious in weight management, since they possess greater thermogenic qualities relative to long chain triacylglycerols; however, MCT may also increase circulating lipid concentrations and risk of cardiovascular disease. **Objective:** Our objective was to examine the effect of a diet supplemented with a functional oil (FctO) composed of energy expenditure-enhancing MCT (50% of fat), cholesterol-lowering phytosterols (22mg/kg body weight) and triacylglycerol-suppressing n-3 fatty acids (5% of fat), vs a beef tallow based control (C) diet, on plasma lipid and aminothiol concentrations. **Design:** In a randomized, single-blind, crossover design, and inpatient trial, 17 overweight women consumed each oil as part of a controlled, supervised, energy-adjusted diet for 27 days, with 4 or 8 weeks of washout between phases. **Results:** Mean body weight decreased ($P<0.01$) during both FctO (-0.87±0.16kg) and C (-0.84±0.22kg) dietary periods. Plasma total cholesterol concentration was lower ($P<0.01$) on FctO (4.37±0.20mmol/L) compared to C (4.80±0.20mmol/L), by 9.0%. LDL cholesterol was also lower ($P<0.01$) following FctO (2.39±0.15mmol/L) vs C (2.86±0.16mmol/L), representing a 16.4% difference. Circulating HDL cholesterol and triacylglycerol remained unaffected by treatment, though HDL:LDL and HDL:total cholesterol ratios increased ($P<0.05$) by 19.5% and 9.4% following FctO. Plasma total homocysteine remained unchanged with FctO but decreased ($P<0.05$) with C, hence higher ($P<0.05$) endpoints were observed with FctO (6.95±0.33umol/L) vs C (6.27±0.28umol/L). Plasma glutathione increased ($P<0.05$) by 0.44umol/L with FctO supplementation.

**Conclusion:** Consumption of a functional oil composed of MCT, phytosterols and n-3 fatty acids for 27 days improves the overall cardiovascular risk profile of overweight women.

## 5.2. Introduction

Obesity is an independent risk factor for cardiovascular disease (CVD), the most common cause of mortality and morbidity in North America (Health Canada, 1997; National Institutes of Health, 1998). Substitution of medium chain triacylglycerols (MCT) for conventional dietary fats has been suggested as beneficial to weight management, since MCT increase energy expenditure and fat oxidation relative to long chain triacylglycerols (LCT) (Hill et al, 1989; Scalfi et al, 1991; Dulloo et al, 1996; White et al, 1999). MCT are rapidly absorbed through the portal circulation (Swift et al, 1990), subsequently oxidized to carbon dioxide (Johnson et al, 1990), and converted to long chain fatty acids in the liver (Carnielli et al, 1994), thus avoiding deposition in peripheral tissues. Animal studies have indeed shown substantial weight loss following MCT consumption (Lavau and Hashim, 1978; Geliebter et al, 1983; Simon et al, 2000), although this effect has yet to be observed thus far in humans (Yost and Eckel, 1989; Temme et al, 1997).

Possible benefits of MCT consumption on energy expenditure may be offset by their undesirable effects on circulating cholesterol and triacylglycerol (TAG) concentrations, both of which are important risk factors for CVD. Long-term feeding of caprylic (8:0) and capric (10:0) acids, MCT with greater potential for energy metabolism modulation, has resulted in plasma cholesterol concentrations higher than those of polyunsaturated fatty acids (PUFA) (Cater et al, 1997; Tsai et al, 1999; Asakura et al,

2000), lower than those of 12:0 (Tsai et al, 1999), and intermediate between those of myristic and oleic acids (Temme et al, 1997). Conversely, some researchers have reported 8:0 and 10:0 to be similarly cholesterol-raising as palm oil (Wardlaw et al, 1995; Cater et al, 1997) and butter (Wardlaw et al, 1995). With respect to effects on TAG, short-term MCT supplementation has demonstrated a 3-fold increase following overfeeding (Hill et al, 1990), and a 42% increase following weight-maintenance feeding (Swift et al, 1992), in comparison to soybean oil. However, long-term MCT feeding has resulted in unchanged fasting TAG, as compared to LCT feeding (Wardlaw et al, 1995; Cater et al, 1997; Temme et al, 1997; Tsai et al, 1999; Asakura et al, 2000).

Phytosterols have been shown to block absorption of dietary and endogenously-derived cholesterol from the gut, while being only minimally absorbed themselves (Jones et al, 1997). Daily consumption of moderate quantities of phytosterols, 0.7-3.2g, has been shown to consistently reduce plasma total cholesterol by 5-13%, and LDL cholesterol by 7-16%, in both hyper- (Miettinen et al, 1995; Jones et al, 1999; Hendriks et al, 1999; Jones et al, 2000) and normocholesterolemic (Pelletier et al, 1995; Hendriks et al, 1999; Tammi et al, 2000) individuals, without affecting HDL cholesterol or TAG concentrations. Plant sterol use has demonstrated excellent tolerability and absence of adverse events, even in children as young as 6 years of age (Tammi et al, 2000).

Alpha-linolenic acid (ALA), a n-3 fatty acid found in flaxseed oil, has been shown to undergo conversion (Cunnane et al, 1993; Mantzioris et al, 1994; Layne et al, 1996) to the potent hypotriacylglycerolemic (Harris, 1997) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in tissue in vivo, thus providing a rationale for suppression of plasma TAG with ALA feeding. In fact, 2 weeks of ALA feeding have reduced fasting

TAG concentrations by 22-24% (Singer et al, 1986), although unchanged concentrations have also been reported (Cunnane et al, 1993; Layne et al, 1996).

We hypothesized that consumption of MCT along with cholesterol-lowering phytosterols and TAG-suppressing n-3 PUFA would prevent undesirable increases in blood lipid concentrations, thus allowing MCT use in weight management. The main objective of the study was to evaluate the effect of this functional oil (FctO), vs beef tallow as control (C), on circulating lipids, as well as fatty acid metabolism.

Plasma total homocysteine (Hcy), an established risk factor for CVD, is associated with circulating cholesterol (Stampfer et al, 1992; Anderson et al, 2000), and has been observed to decrease following fish oil supplementation (Olszewski and McCully, 1993). Moreover, Hcy exposure, and saturated fat and cholesterol intake, have been reported to facilitate the development of atherogenic lesions in rabbits (McCully et al, 1990), and to work synergistically in elevating plasma total Hcy, cholesterol and TAG concentrations in rats (Zulli et al, 1998). Therefore, we hypothesised that consumption of the FctO would alter Hcy, through influence of n-3 PUFA or as a result of cholesterol modification. The secondary study objective was thus to measure plasma aminothiols, in relation to CVD risk, and Hcy modulation following dietary fat modification.

## 5.3. Subjects and Methods

**Subjects** Twenty-two healthy overweight women were recruited from the surrounding community through newspaper advertising. Enrolled subjects had BMI > 25kg/m$^2$, plasma total cholesterol concentration $\leq$ 7.0mmol/L and total circulating TAG concentration $\leq$ 3.0mmol/L, as measured at screening after 12 hours of fasting and 24 hours of alcohol abstinence. Subject body weights were required to have been stable

(±5%) for at least 3months prior to study entrance. All subjects were screened through interview for reported absence of existing chronic illnesses including diabetes, hypertension, cardiac, hepatic, renal, and gastrointestinal dysfunction. Other exclusion criteria included use of lipid-lowering drugs, beta-blockers or diuretics, and personal history of CVD. Those reporting exercise at a frequency of $\geq$ 5 times per week, or ongoing pregnancy or lactation were excluded. Prior to study onset, subjects received a complete description of the protocol before signing a consent form in presence of the study investigators. The experimental protocol was approved by the Human Ethical Review Committee of the Faculty of Agriculture and Environmental Sciences for the School of Dietetics and Human Nutrition at McGill University.

**Study Protocol** The study was a randomized, single-blind, controlled, inpatient clinical trial. A crossover design was used, with two 27-day dietary feeding cycles, separated by 4 or 8 weeks of washout, during which subjects resumed their habitual diets. Subjects were randomly allocated to receive one of two treatment sequences, with a balanced number of subjects assigned to each dietary treatment per phase. Subjects were inpatients at the Mary Emily Clinical Nutrition Research Unit (CNRU) at McGill University during supplementation periods. Participants were allowed to leave the facility between meals for work or other approved purposes, but were expected to remain at the unit after the evening meal and overnight. Meals were consumed under supervision at the CNRU, however, under unusual circumstances, study coordinators permitted meals to be packed and consumed outside the unit; this occurred for < 2% of meals. Although physical activity was not encouraged, exercise facilities were available at the unit. Subjects were instructed to report their daily physical activities in a journal during the

first phase of the study, and to reproduce the same intensity and duration of activity on corresponding days of the second phase. A physician familiar with the study protocol and diets was available throughout the trial in case subjects experienced discomfort. Fasting blood samples were collected on days 1, 26 and 28 of each dietary phase; d26 accounting for day-to-day variation in circulating cholesterol. Since the same menu was assigned to corresponding days across phases, foods consumed preceding day 26 blood draws were identical, except for treatment fat, and similarly for day 28 blood draws. To measure fatty acid excretion, total fecal samples were collected for 3 days at midpoint of each phase, a period chosen to allow for sufficient adaptation to experimental diets. Using the present protocol, each subject was tested during the same phase of her menstrual cycle for corresponding time points across study periods.

**Diets** Experimental diets consisted of prepared North American solid foods, precisely weighed to the nearest 0.5g, and based on a 3-day rotating cycle menu (**Table 5.3.1**). Diets were served as 3 isoenergetic meals per day, and provided 45% of energy as carbohydrate, 15% as protein, and 40% as fat, of which 75% was delivered as treatment fat and the remaining 25% was found in the basal diet food items. Treatment fat of each diet, FctO or C fat, was directly incorporated into food items during meal preparation and cooking to effect blinding (Table 5.3.1). The FctO consisted of 3 major lipid components: MCT, phytosterols and n-3 PUFA. A combination of MCT oil (50% of fat) (Neobee 1053; Stepan Company, Northfield, IL, US), butter (5% of fat) and coconut oil (5% of fat) comprised the MCT portion of the FctO. Tall oil phytosterols, with major components sitosterol, campesterol and sitostanol, in the unesterified form (Forbes

**Table 5.3.1:** Cycle menu of the basal diet

| Meal | Day 1 | Day 2 | Day 3 |
|---|---|---|---|
| Breakfast | Orange juice<br>1% milk<br>Fruit and fibre cereal<br>Vegetable omelette *<br>Blueberry muffin * | Orange juice<br>Fruit salad<br>Cottage cheese<br>English muffin *<br>Scrambled eggs * | Orange juice<br>Low-fat fruit yoghurt<br>French toast *<br>Maple syrup |
| Lunch | Kiwi-orange juice<br>Broccoli soup<br>Ham and cheese sandwich *<br>Yoghurt cake*<br>Fruit sauce | Apple juice<br>Turkey meat loaf<br>Tomato sauce<br>Cooked carrots and green beans<br>Mashed potatoes *<br>Oatmeal cookies * | Apple juice<br>Tomato and cucumber salad *<br>Homemade pizza on pita bread *<br>Carrot cake *<br>Low-fat icing |
| Dinner | Banana-orange juice<br>Roasted turkey breast<br>Cranberry sauce<br>Cooked carrots<br>Mashed potatoes *<br>Ice milk<br>Strawberries | Caffeine-free diet soda<br>Beef stir-fry *<br>Rice *<br>Frozen yoghurt<br>Ginger snap cookies * | Caffeine-free diet soda<br>Raw celery and carrot sticks<br>French bread *<br>Spaghetti with meat sauce *<br>Low-fat mozzarella cheese<br>Date square * |

* Denotes food items to which treatment fat, either functional oil or control oil, has been added.

phytosterols; Forbes Medi-Tech Inc, Vancouver, BC, Canada), were administered at a concentration of 2.2mg/kg body weight per day (average daily intake=1.81g). Based on a mean daily FctO intake of 86.2g, the mean concentration of phytosterols in the FctO was 2.1% (wt/wt). Since plant sterols were dispersed in fat, and the latter was incorporated to meals during cooking, sufficient solubilization and intestinal availability were ensured. The n-3 PUFA portion of the oil was provided by flaxseed oil (5% of fat), which was incorporated into meals after heating was complete in order to minimize peroxidation of unsaturated fatty acids. Olive oil (10% of fat) was also present in the FctO to approach the proportion of monounsaturated fatty acids to that of C. The intake of each fat component was equally distributed over the three daily meals. Treatment fat for the C diet was composed exclusively of beef tallow. Forty-seven percent of fatty acids in the FctO diet had $\leq$ 12 carbons, while 66% of those in the C diet had $\geq$ 18 carbons (**Table 5.3.2**). Non-fat constituents were identical across diets.

Since our objective was to provide a weight-maintaining diet, the nutrient intake was adjusted to individual subject energy requirements using the Mifflin equation (Mifflin et al, 1990), to which an activity factor of 1.7 was multiplied to compensate for additional energy needs of active adults (Shils et al, 1999). The different energetic contribution of MCT and LCT, 34 and 38 kJ/g respectively, were accounted for in the calculation of energy intake, in order that FctO and C diets be isoenergetic. During the first week of phase 1, energy intake was readjusted, if needed, by increases or decreases of 2% following losses or gains in body weight, in order to re-establish energy balance. Energy intake was fixed thereafter and was identical during both dietary treatment cycles

**Table 5.3.2:** Fatty acid composition of experimental diets [1]

| Fatty acid | Control diet | Functional oil diet |
|---|---|---|
| | *% of total identified fatty acids* | |
| 8:0 | Trace | 19.4 ± 2.0 |
| 10:0 | 0.2 ± 0.1 | 23.6 ± 2.3 |
| 12:0 | 0.3 ± 0.1 | 3.9 ± 0.6 |
| 14:0 | 3.4 ± 0.4 | 2.6 ± 0.5 |
| 16:0 | 26.1 ± 0.9 | 10.1 ± 1.1 |
| 18:0 | 20.3 ± 1.1 | 3.8 ± 0.6 |
| 18:1n-9 | 38.5 ± 1.6 | 23.6 ± 3.5 |
| 18:2n-6 | 6.4 ± 1.6 | 7.1 ± 1.6 |
| 18:3n-3 | 0.8 ± 0.1 | 4.6 ± 1.3 |
| Σ SFA | 50.9 ± 0.5 | 63.8 ± 1.0 |
| Σ MUFA | 41.9 ± 0.4 | 24.4 ± 0.8 |
| P:S ratio | 0.14 ± 0.01 | 0.19 ± 0.01 |
| n-6:n-3 ratio | 7.2 ± 0.3 | 1.5 ± 0.1 |

[1] Mean ± SEM composition of 9 meals from the 3-day menu, analyzed in duplicates, thus representing 18 measurements for each diet.

for each subject. Body weight was monitored daily before breakfast during feeding periods to assess change in body weight. No extra food was allowed between meals, except for decaffeinated, energy-free carbonated beverages and herbal teas, which were obtained from kitchen staff. One black coffee was allowed per day at breakfast. Alcoholic beverages were prohibited during dietary phases. Health Canada recommendations (Health Canada, 1990) were met for all vitamins, minerals, fibre, carbohydrate subcomponents, and essential fatty acids. The nutrient content of the diet, other than fatty acids, was determined with Food Processor (Esha Research, Salem, OR, US), a computerized dietary analysis program equipped with a Canadian database.

**Analyses** *Blood Lipid Measurement* Blood samples were drawn after a 12-hour overnight fast, and at least 24 hours of alcohol abstinence (for day 1), and collected in EDTA-containing Vacutainer® (BD, Franklin Lakes, NJ, US) tubes. Samples were immediately centrifuged at room temperature using a table top centrifuge for 15 minutes at 250Xg, and resulting plasma and red blood cell subfractions were refrigerated, separated within an hour of collection, and stored at –80°C until analysis. All tubes were coded by an external party to blind investigators for analysis and data compiling procedures. Plasma total and HDL cholesterol, and TAG concentrations, were analyzed in quadruplicate with standardized reagents using a VP Autoanalyser (Abbott Laboratories, North Chicago, IL, US). Calibration of the machine prior to each run was performed as per the standardization protocol of the Canadian Reference Laboratory (1996, Vancouver, BC, Canada), which involves direct comparison with fresh specimen samples. Certification for traceability using this method was maintained through the National Reference System. Measurement of HDL cholesterol in plasma was done after

precipitation of apolipoprotein B with dextran sulfate and magnesium chloride (Warnick et al, 1982). LDL cholesterol concentrations were calculated using the Friedewald equation (Friedewald et al, 1972). Coefficients of variation for replicate analyses of total, HDL cholesterol and TAG concentrations were 1.4%, 2.3%, and 3.1%, respectively.

*Homocysteine and Other Aminothiols Measurements* Total Hcy, cysteine, cysteinylglycine and glutathione were measured in fasting plasma samples using a modified isocratic high-performance liquid chromatography method with fluorescence detection of derivatized aminothiols, as previously described (Durand et al, 1996). Aminothiols were reduced and released from proteins by incubation with tri-*n*-butylphosphine (10%) in dimethylformamide for 30min at 4°C. Proteins were then precipitated with 0.6mol/L cold perchloric acid containing EDTA. Derivatization was done with ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate (SBD-F) in sodium hydroxide and potassium borate at 60°C for 60min. After cooling on ice, 10μL of samples were injected into a high-performance liquid chromatograph (System Gold; Beckman, Fullerton, CA, US) equipped with an analytical reverse phase column (C18ODS, 250X4.6mm, Beckman). Isocratic conditions were used with a 0.45:2 acetate:acetic acid buffer, pH 4.0, and a flow rate of 1.3mL/min for 25min. Fluorescence detection was carried with a 305-395nm excitation filter and a 430-470nm emission filter. Acetyl-cysteine was used as internal standard to allow quantification. The day-to-day coefficients of variation were 2.5%, 6.5%, 3.2%, and 6.6% for Hcy, cysteine, cysteinylglycine, and glutathione, respectively.

*Fatty Acid Composition Determination* Individual meals in each 3-day cycle diet, red blood cells on d1 and d28, and 3-day total fecal samples were analysed in duplicate for fatty acid composition by gas-liquid chromatography after lipid extraction (Folch et al, 1957), sodium hydroxide saponification (Al Makdessi et al, 1985) and boron-trifluoride methylation (Al Makdessi et al, 1985). Briefly, 3g of homogenized food or freeze-dried feces, or 2g of red blood cells, were agitated with methanol at 55°C for 15min. Following choloroform:hexane (1:4) extraction, non-polar phases were evaporated under nitrogen gas. The residue obtained was saponified for 15min at 80°C with 0.5mol/L sodium hydroxide in methanol. After acidification with sulphuric acid, hexane extraction was performed to recover lipids. Fatty acids were methylated with boron trifluoride methanol:hexane:methanol (7:6:7, v/v/v) for 1 hour at 100°C, and the resulting fatty acyl methyl esters (FAME) were extracted with hexane and sealed in crimp vials. Prior to lipid extraction of red blood cell samples, 0.1% butylated hydroxytoluene (BHT) was added to minimize peroxidation of long chain PUFA.

Derivatized samples were injected via an autoinjector into a gas chromatograph (HP 5890 Series II; Hewlett Packard, Palo Alto, CA) equipped with a flame ionization detector, and a 30m capillary column, with 0.2mm internal diameter and 0.25μm film thickness (SP2330; Supelco, Bellefonte, PA). The injector and detector temperatures were 200°C and 210°C respectively, and the split ratio was 100:1. A multiple ramp temperature program was used to separate individual FAME, and identification was done using relative retention times of authentic standards (Supelco). Fatty acid composition of each diet is reported in Table 5.3.2. MCT oil was determined to be 0.1% 6:0, 49.1% 8:0, 49.8% 10:0 and 1.0% 12:0. Coefficients of variation for replicate food fatty acid

analyses, fatty acid content of different meals, and menu cycle days were 12.3%, 6.6%, and 2.6%, respectively.

Total fecal samples collected for 3 days at midpoint of each phase were thawed, weighted, diluted with 2 volumes of water and homogenized for 10min using a shaking arm apparatus. Samples were then freeze dried to remove all trace of water. For fatty acid analyses, dry fecal samples collected within a phase were pooled based on their relative contribution to total fecal mass. One milligram of the internal standard 17:0 was added to samples for quantification of fecal fatty acids. Methylation was done using 1mL of boron trifluoride methanol. The injector and detector temperatures were 250°C.

**Statistical Methods** Plasma lipid and aminothiol concentrations, body weight and fatty acid composition data have been reported as mean±SEM. Dietary fatty acid composition has been presented descriptively but has not been analyzed statistically. ANOVA was carried out using a mixed model for repeated measures, with factors of phase, sequence, diet, time, time-by-phase interaction and time-by-diet interaction. Paired Student's *t* test was then applied to the model to compare baseline and endpoint values within dietary phases. Scheffe's adjustment was performed to identify significant differences between C and FctO diets at corresponding times. Pearson's product-moment correlations were carried out on residuals computed from the model, in order to assess specific relationships between outcome measures. The level of significance for rejection of the null hypothesis was set at $P<0.05$. Version 8.0 of SAS software (SAS Institute, Cary, NC, US, 1999) was used to perform all statistical analyses.

## 5.4. Results

Twenty-two subjects enrolled, and 17 subjects completed both phases of the trial. Three subjects withdrew consent for personal reasons and 2 subjects were unable to tolerate the beef tallow-containing C diet. The age of women who completed the trial ranged from 21 to 65yr, with a mean of 44±4yr, and BMI ranged from 26 to 41kg/m$^2$ with a mean of 32±1kg/m$^2$. Mean fasting plasma total cholesterol and TAG concentration at screening were 5.12±0.17mmol/L and 1.57±0.14mmol/L, respectively. Four subjects were smokers, and 8 were post-menopausal. Mean energy intake was 10.28±0.31MJ/d (2458±73kcal/d) and mean phytosterol intake was 1.81±0.06g/d.

The FctO diet was generally well tolerated, except for minor gastrointestinal discomfort and occasional nausea during the first few days of supplementation, which did not require medical intervention or lead to any subject withdrawing from the study protocol. Some subjects reported that the C fat had an unpleasant smell, taste and/or aftertaste. The FctO was generally preferred by subjects and was not reported to have any particular smell, taste or texture. Even though most subjects were able to detect a difference in the diets' organoleptic properties, they could not successfully identify the C or FctO diets when questioned. Subjects consumed all food provided and reported increased post-meal satiety, due to larger meal size compared to their habitual diets. During the first week of phase 1, energy intake was increased by 2% for 3 subjects (one on C and 2 on FctO) to compensate for weight loss, and was reduced by 2% for 2 subjects (one on each diet) to compensate for weight gain.

Baseline values of all variables were not statistically different between diets. Mean body weight decreased ($P$<0.01) during both FctO (82.46±2.77kg on d1 and 81.59±2.75kg on d28) and C (82.14±2.65kg and d1 to 81.30±2.75kg on d28) phases, but

no differential weight loss was discerned between dietary cycles (-0.87±0.16kg on FctO vs -0.84±0.22kg on C). Individual subject variations in body weight indicated that 8 out of 17 subjects experienced a larger decrease on the FctO vs C diet. Changes in BMI paralleled those of body weight, with mean reductions ($P<0.01$) of 0.33±0.06kg/m$^2$ on FctO and 0.33±0.09kg/m$^2$ on C.

Plasma and red cell values for one subject were excluded from means on d1 of the FctO phase, as improper fasting was suggested by TAG concentrations well above the subject's normal range. Mean plasma lipid concentration on days 1, 26 and 28 of each supplementation period, and percent change over time are presented in **Table 5.4.1.** Mean total cholesterol concentration remained unchanged on C but declined by 0.24mmol/L on d26 of the FctO diet, while the decline on d28 was marginally significant. An average 4.8% decrease in total cholesterol was achieved over a month of FctO supplementation when compared to baseline. Time-by-diet interactions were identified for total cholesterol on d26 and d28. Mean endpoint (average of d26 and d28) total cholesterol concentration was lower on FctO vs C (4.37±0.20mmol/L vs 4.80±0.20mmol/L), by a difference of 9.0%. **Figure 5.4.1** shows the individual subject variation in plasma total cholesterol between endpoints of each diet. Consistently lower endpoint values for total cholesterol were observed for all 17 subjects on FctO as compared to C. Percent change in total cholesterol from baseline showed large between-subject variability, reflecting a range of dietary intakes. The quartile of subjects having the least favourable response to the C diet (n=4) had a mean 15.3% increase in total cholesterol, while the quartile of subjects having the most favourable response (n=4) had a mean 12.4% decrease in total cholesterol. Similarly for the FctO diet, a mean 9.1%

increase in total cholesterol was found in the quartile of subjects having the least favourable response (n=4), while a 14.5% decrease was found in the quartile with the most favourable response (n=4).

Mean plasma LDL cholesterol concentration decreased by 0.25mmol/L and 0.28mmol/L on d26 and d28 of the FctO diet, while no significant change was observed on the C diet (Table 5.4.1). A mean 10.4% decline in LDL cholesterol was observed on the FctO phase. Lower LDL cholesterol values were found on d26, and d28 of the FctO diet, when compared to the C diet. Mean endpoint LDL cholesterol concentration was 2.39±0.15mmol/L on FctO and 2.86±0.16mmol/L on C. Substituting FctO for C thus resulted in lower LDL cholesterol values, with a mean difference of 16.4%. **Figure 5.4.2** shows individual subject variation in LDL cholesterol concentration between d26/28 of each diet. Fifteen out of 17 subjects experienced lower endpoint LDL cholesterol concentration on the FctO diet compared to the C diet. Two subjects had higher endpoint LDL cholesterol values on the FctO diet, with differences of +0.09mmol/L and +0.03mmol/L, whereas the mean difference for all subjects was -0.46±0.08mmol/L. Percent change in LDL cholesterol over time was greatly variable between subjects. The quartile of subjects having the least favourable response to the C diet (n=4) had a mean 26.5% increase in LDL cholesterol, while the quartile of subjects having the most favourable response (n=4) had a mean 20.8% decrease. For the FctO diet, a mean 13.8% increase in LDL cholesterol was experienced by the quartile of subjects with the least favourable response (n=4), and LDL cholesterol decreased by 28.1% for those having the most favourable response (n=4).

Since HDL cholesterol remained unchanged with either dietary supplementation, the protective HDL:LDL and HDL:total cholesterol ratios increased by 19.5% and 9.4%,

respectively, on the FctO diet (Table 5.4.1). Although there was no significant time-by-diet interaction, a main effect of diet was noted for these ratios. Percent change in the atherogenic ratio of LDL:HDL cholesterol differed ($P < 0.05$) across diets, with mean variations of +6.4±6.6% on C and −12.1±5.2% on FctO diets. Likewise, percent decline in total:HDL cholesterol ratio on the FctO diet (-7.0±3.3%) differed ($P<0.05$) from the increase obtained on the C diet (+2.4±4.2%). Compared to baseline, absolute values for LDL:HDL cholesterol on d26 and d28 of the FctO phase were marginally ($P=0.0542$) and significantly ($P<0.05$) decreased, respectively. No significant time-by-diet interaction was identified for LDL:HDL cholesterol, but a main effect of diet ($P<0.05$) was noted. Only marginally significant main effect of diet ($P=0.0593$) and within phase comparisons ($P<0.08$) were observed for absolute total:HDL cholesterol values.

Circulating plasma TAG concentration did not show statistical difference for diet effects, although it had a tendency to rise on the FctO diet and to decline on the C diet. A marginally significant decrease from baseline was noted on d26 of the C diet. High variability in TAG values is confirmed by a significant difference between d26 and d28 on the FctO diet. Eleven subjects out of 17 had higher endpoint TAG on the FctO vs C diet. Percent changes in body weight and BMI were not correlated with changes in lipoprotein cholesterol, but were correlated with changes in TAG concentration ($r=0.554$, $P<0.001$ for weight, and $r=0.544$, $P<0.01$ for BMI). Separate dietary correlation analysis revealed that the association between changes in TAG and body weight was only present in the C diet ($r=0.705$, $P<0.01$) but nonexistent in the FctO diet, and likewise for the association between changes in TAG and BMI ($r=0.697$, $P<0.01$ on C).

Plasma aminothiol concentration on days 1, 26 and 28, and percent change over time are presented in **Table 5.4.2**. Hcy concentration decreased on the C diet by 5.4%,

while a marginal increase from baseline was seen on d26 of the FctO diet. Higher Hcy was observed on FctO vs C for d26, d28, and percent change. Mean endpoint Hcy values were higher on FctO (6.95±0.33μmol/L) vs C (6.27±0.28μmol/L), with a difference of 10.8%. Individual subject variation in endpoint plasma total Hcy concentration between diets is shown in **Figure 5.4.3**. All subjects consistently experienced higher endpoint total Hcy on the FctO vs C diet. Cysteine concentration remained unchanged, although percent variations were marginally different between diets (Table 5.4.2). Cysteinylglycine remained unchanged with C, but decreased by 1.77μmol/L on d26 of FctO. Glutathione was increased from baseline on d28 of the FctO diet by 0.44μmol/L but did not vary with C. Correlation analysis did not reveal an association between changes in Hcy and cholesterol.

Relative fatty acid composition data in red blood cells on d1 and d28 of each dietary phase are presented in **Table 5.4.3**. Fatty acids with chain lengths of 6, 8, 10 and 12 carbons were not detected in this tissue. C diet consumption decreased the proportion of 14:0, 16:0, 18:3n-3 and EPA, and increased the proportion of 18:0, 20:4n-6 and 22:4n-6 in red blood cells, as compared to baseline. The sum of n-6 fatty acids increased by 1.2%, while the n-6:n-3 ratio increased by 7.9% after 27 days of beef tallow feeding. FctO diet consumption increased tissue 18:3n-3, EPA and the sum of n-3 fatty acids, and decreased 18:1n-9, the sum of monounsaturated fatty acids, and the ratio of n-6:n-3 fatty acids by 12.2%. DHA, 22:5n-3 and the sum of saturated fatty acids remained unchanged on either diets. Mean endpoint value on FctO was higher for 14:0, 16:0, 18:3n-3, EPA and the sum of n-3 fatty acids, and lower for the n-6:n-3 ratio, in comparison to C. Changes in red blood cell fatty acids were not associated to those of plasma cholesterol or lipoproteins. However, changes in circulating TAG were positively associated with those

of red cell 14:0 (r=0.348, $P<0.05$) and n-6:n-3 ratio (r=0.356, $P<0.05$), and negatively associated with the sum of n-3 fatty acids (r=-0.431, $P<0.05$). These relationships were absent in the FctO diet, while some retained significance in the C diet (TAG and 14:0: r=0.483, $P<0.05$, TAG and sum of n-3 fatty acids: r=-0.594, $P<0.05$). Percent changes in tissue EPA and sum of n-3 fatty acids were not correlated with TAG variations.

Fecal fatty acid concentration per weight of dry fecal matter is shown in **Table 5.4.4**. Fecal fatty acid data were excluded from means for one subject due to sample loss during extraction. No trace of 6:0, 8:0 or 10:0 was detected in feces. The fecal fatty acid content in each phase generally reflected corresponding dietary intake. Lower concentration of total fatty acids in feces was found on FctO vs C. Changes in plasma cholesterol were not correlated with fatty acid excretion. Changes in TAG were negatively correlated with the excretion of 18:0 (r=-0.540, $P<0.05$), 18:3n-3 (r=-0.547, $P<0.05$), and positively correlated with total fatty acid excretion (r=0.506, $P<0.05$), but these relationships were only present in the C diet.

**Table 5.4.1:** Effect of experimental diets on plasma lipid concentrations [1]

| Plasma lipid and study day | | Control diet | Functional oil diet |
|---|---|---|---|
| Total cholesterol [2] | (mmol/L) | | |
| Day 1 | | 4.77 ± 0.17 | 4.58 ± 0.21 |
| Day 26 | | 4.76 ± 0.21 | 4.34 ± 0.20 [3, 4] |
| Day 28 | | 4.84 ± 0.21 | 4.40 ± 0.19 [5, 6] |
| Change | (%) | +0.8 ± 2.7 | -4.8 ± 2.5 [7] |
| LDL cholesterol [2] | (mmol/L) | | |
| Day 1 | | 2.76 ± 0.12 | 2.66 ± 0.15 |
| Day 26 | | 2.84 ± 0.17 | 2.41 ± 0.16 [3, 4] |
| Day 28 | | 2.87 ± 0.16 | 2.38 ± 0.14 [3, 6] |
| Change | (%) | +4.0 ± 5.0 | -10.4 ± 4.3 [4] |
| HDL cholesterol | (mmol/L) | | |
| Day 1 | | 1.33 ± 0.07 | 1.30 ± 0.08 |
| Day 26 | | 1.32 ± 0.07 | 1.31 ± 0.08 |
| Day 28 | | 1.32 ± 0.07 | 1.33 ± 0.08 |
| Change | (%) | +0.3 ± 3.7 | +3.3 ± 2.7 |
| HDL:LDL cholesterol ratio [8] | | | |
| Day 1 | | 0.490 ± 0.029 | 0.495 ± 0.026 |
| Day 26 | | 0.483 ± 0.033 | 0.571 ± 0.036 [3] |
| Day 28 | | 0.478 ± 0.030 | 0.581 ± 0.037 [3] |
| Change | (%) | +1.9 ± 8.4 | +19.5 ± 6.7 |
| HDL:total cholesterol ratio [9] | | | |
| Day 1 | | 0.279 ± 0.012 | 0.281 ± 0.010 |
| Day 26 | | 0.278 ± 0.011 | 0.304 ± 0.012 [3] |
| Day 28 | | 0.274 ± 0.010 | 0.304 ± 0.013 [3] |
| Change | (%) | +0.7 ± 4.7 | +9.4 ± 3.7 |
| Total triacylglycerols | (mmol/L) | | |
| Day 1 | | 1.48 ± 0.12 | 1.36 ± 0.15 |
| Day 26 | | 1.32 ± 0.13 [10] | 1.35 ± 0.14 |
| Day 28 | | 1.42 ± 0.13 | 1.48 ± 0.13 [11] |
| Change | (%) | -4.1 ± 6.3 | +7.3 ± 4.1 |

[1] Mean ± SEM; n=17 women for each period, except for day 1 and percent change on the functional oil diet (n=16).

[2, 8, 9] Significant main effect of diet, [2] $P<0.0001$, [8] $P<0.01$, [9] $P<0.05$.

[3] Significantly different from day 1 within dietary phase, $P<0.05$.

[5, 10] Trend toward significant difference from day 1 within dietary phase, [5] $P=0.0693$, [10] $P=0.0769$.

[11] Significantly different from day 26 within dietary phase, $P<0.05$.

[4, 6] Significantly different from the control diet, [4] $P<0.05$, [6] $P<0.01$.

[7] Trend toward significant difference from the control diet, [7] $P=0.0568$.

**Table 5.4.2:** Effect of experimental diets on plasma aminothiol concentrations [1]

| Aminothiol | | Control diet | Functional oil diet |
|---|---|---|---|
| Homocysteine [2] | (μmol/L) | | |
| Day 1 | | 6.68 ± 0.33 | 6.55 ± 0.33 |
| Day 26 | | 6.30 ± 0.30 [3] | 6.97 ± 0.31 [4, 5] |
| Day 28 | | 6.24 ± 0.29 [3] | 6.92 ± 0.36 [6] |
| Change | (%) | -5.4 ± 2.2 | 4.6 ± 2.4 [6] |
| Cysteine | (μmol/L) | | |
| Day 1 | | 225 ± 7 | 217 ± 9 |
| Day 26 | | 221 ± 7 | 228 ± 9 |
| Day 28 | | 220 ± 8 | 228 ± 9 |
| Change | (%) | -1.7 ± 1.8 | 3.7 ± 1.9 [7] |
| Cysteinylglycine | (μmol/L) | | |
| Day 1 | | 24.9 ± 1.4 | 24.9 ± 1.3 |
| Day 26 | | 24.3 ± 1.4 | 23.1 ± 1.0 [3] |
| Day 28 | | 24.1 ± 1.1 | 23.9 ± 0.9 |
| Change | (%) | -2.0 ± 3.0 | -3.4 ± 1.9 |
| Glutathione | (μmol/L) | | |
| Day 1 | | 3.10 ± 0.32 | 2.93 ± 0.36 |
| Day 26 | | 3.24 ± 0.25 | 3.28 ± 0.24 |
| Day 28 | | 3.22 ± 0.29 | 3.37 ± 0.26 [3] |
| Change | (%) | 8.6 ± 6.5 | 25.4 ± 10.8 |

[1] Mean ± SEM; n=17 women for each period, except for day 1 and percent change on the functional oil diet (n=16).
[2] Significant main effect of diet, $P<0.0001$.
[3] Significantly different from day 1 within dietary phase, $P<0.05$.
[4, 6] Significantly different from the control diet, [4] $P<0.05$, [6] $P<0.01$.
[5] Trend toward significant difference from day 1 within dietary phase, $P=0.0804$.
[7] Trend toward significant difference from the control diet, $P=0.0675$.

**Table 5.4.3:** Fatty acid composition of red blood cells at beginning and end of experimental diet supplementation [1]

| Fatty acid | Control diet | | Functional oil diet | |
|---|---|---|---|---|
| | Day 1 | Day 28 | Day 1 | Day 28 |
| | *% of total identified fatty acids* | | | |
| 14:0 [2] | 0.34 ± 0.03 | 0.26 ± 0.02 [3] | 0.34 ± 0.03 | 0.38 ± 0.03 [4] |
| 16:0 [5] | 21.57 ± 0.37 | 19.93 ± 0.30 [6] | 21.15 ± 0.41 | 21.19 ± 0.44 [4] |
| 16:1n-7 | 0.79 ± 0.09 | 0.65 ± 0.05 | 0.67 ± 0.09 | 0.89 ± 0.12 |
| 18:0 | 11.21 ± 0.40 | 12.41 ± 0.33 [3] | 11.13 ± 0.45 | 11.48 ± 0.43 |
| 18:1n-9 | 21.93 ± 0.40 | 21.68 ± 0.38 | 22.19 ± 0.62 | 21.05 ± 0.37 [3] |
| 18:2n-6 | 12.20 ± 0.54 | 11.87 ± 0.45 | 12.17 ± 0.62 | 11.46 ± 0.65 |
| 18:3n-6 | 0.06 ± 0.01 | 0.05 ± 0.01 | 0.07 ± 0.01 | 0.05 ± 0.01 |
| 18:3n-3 [7] | 0.08 ± 0.01 | 0.05 ± 0.01 [3] | 0.08 ± 0.01 | 0.18 ± 0.02 [6, 8] |
| 20:1n-9 | 0.09 ± 0.01 | 0.08 ± 0.01 | 0.08 ± 0.01 | 0.09 ± 0.01 |
| 20:2n-6 | 0.65 ± 0.11 | 0.62 ± 0.08 | 0.63 ± 0.12 | 0.73 ± 0.14 |
| 20:3n-6 | 1.65 ± 0.10 | 1.62 ± 0.09 | 1.69 ± 0.11 | 1.56 ± 0.12 |
| 20:4n-6 [9] | 16.75 ± 0.30 | 17.72 ± 0.32 [3] | 16.16 ± 0.41 | 16.60 ± 0.37 |
| 20:5n-3 [7] | 0.84 ± 0.04 | 0.66 ± 0.06 [10] | 0.80 ± 0.09 | 1.13 ± 0.08 [6, 8] |
| 22:4n-6 [2] | 3.51 ± 0.17 | 4.18 ± 0.24 [3] | 4.81 ± 0.45 | 4.64 ± 0.42 |
| 22:5n-3 | 3.84 ± 0.15 | 3.76 ± 0.19 | 3.72 ± 0.17 | 4.00 ± 0.19 |
| 22:6n-3 | 4.49 ± 0.20 | 4.47 ± 0.16 | 4.30 ± 0.27 | 4.56 ± 0.18 |
| Σ SFA | 33.11 ± 0.37 | 32.60 ± 0.47 | 32.62 ± 0.55 | 33.05 ± 0.67 |
| Σ MUFA | 22.81 ± 0.41 | 22.41 ± 0.41 | 22.94 ± 0.60 | 22.04 ± 0.40 [3] |
| Σ n-6 PUFA | 34.82 ± 0.52 | 36.06 ± 0.47 [10] | 35.53 ± 0.41 | 35.05 ± 0.50 |
| Σ n-3 PUFA | 9.26 ± 0.20 | 8.93 ± 0.31 | 8.90 ± 0.37 | 9.87 ± 0.30 [10, 11] |
| P:S ratio | 1.34 ± 0.03 | 1.39 ± 0.04 | 1.37 ± 0.04 | 1.38 ± 0.05 |
| n-6:n-3 ratio | 3.80 ± 0.12 | 4.10 ± 0.13 [3] | 4.09 ± 0.16 | 3.59 ± 0.10 [12, 4] |

[1] Mean ± SEM; n=17 women for each period, except for day 1 of the functional oil diet (n=16).

[2, 7, 9] Significant main effect of diet, [2] $P<0.01$, [7] $P<0.0001$, [9] $P<0.05$.

[5] Trend toward significant main effect of diet, $P=0.0576$.

[3, 6, 10, 12] Significantly different from day 1 within dietary phase, [3] $P<0.05$, [6] $P<0.0001$, [10] $P<0.01$, [12] $P<0.001$.

[4, 8, 11] Significantly different from the control diet at corresponding time points, [4] $P<0.01$, [8] $P<0.0001$, [11] $P<0.05$.

**Table 5.4.4:** Fatty acid composition of fecal samples obtained from subjects consuming experimental diets [1]

| Fatty acid | Control diet | Functional oil diet |
|---|---|---|
| | *mg fatty acid/g dry feces* | |
| 12:0 [2] | 0.02 ± 0.002 | 0.07 ± 0.01 |
| 14:0 [3] | 0.27 ± 0.02 | 0.22 ± 0.02 |
| 16:0 [4] | 6.28 ± 0.70 | 3.70 ± 0.32 |
| 18:0 [4] | 6.94 ± 1.01 | 3.00 ± 0.53 |
| 18:1n-9 [2] | 5.41 ± 0.28 | 4.05 ± 0.29 |
| 18:2n-6 | 1.38 ± 0.12 | 1.65 ± 0.24 |
| 18:3n-3 | 0.10 ± 0.01 | 0.13 ± 0.02 |
| Total fatty acids [2] | 21.54 ± 1.91 | 14.13 ± 1.17 |

[1] Mean ± SEM; n=16 women in each dietary phase, 3-day total fecal samples collected at mid phase.

[2, 3, 4] Significantly different between diets: [2] $P<0.0001$, [3] $P<0.01$, [4] $P<0.001$.

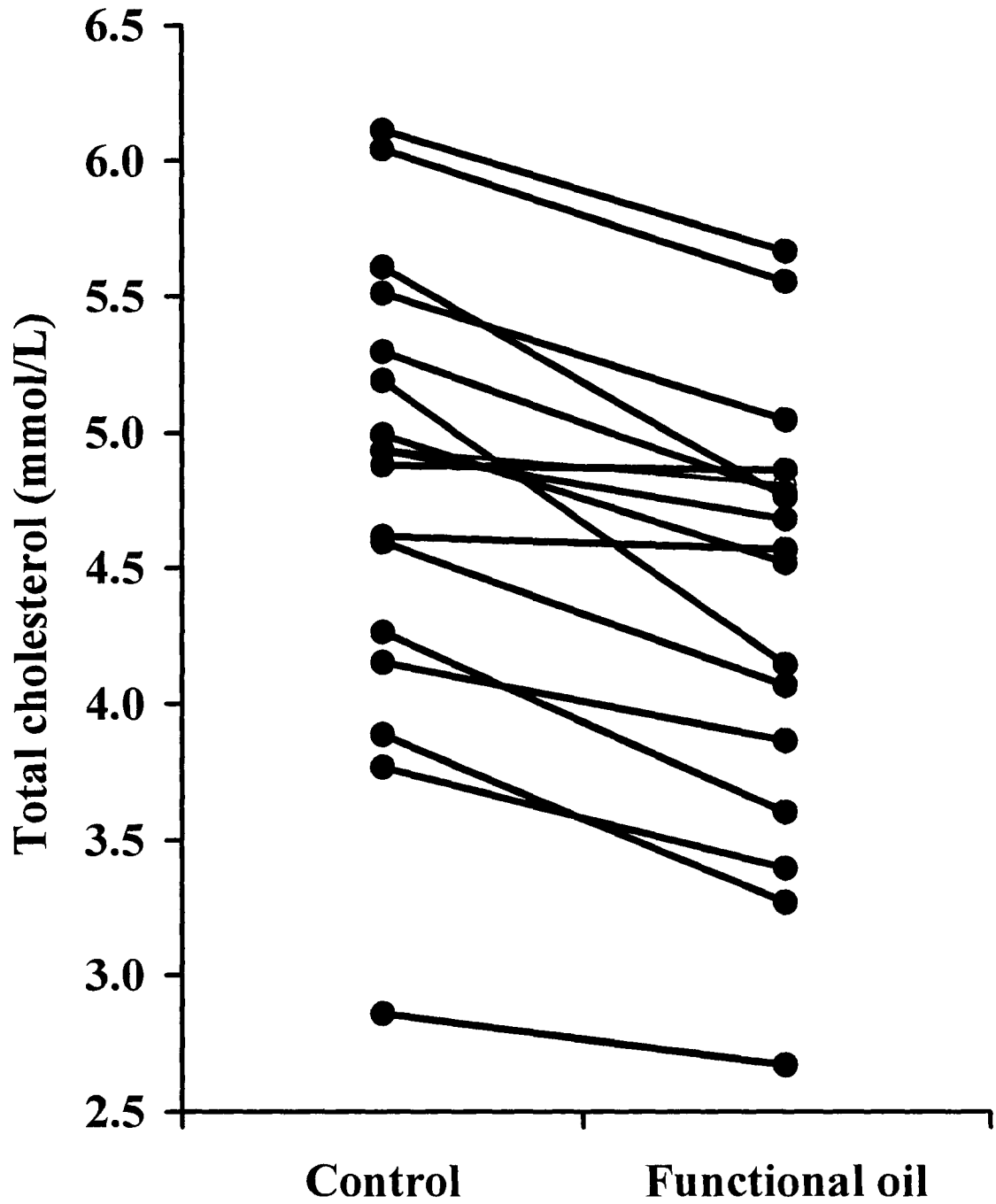


**Figure 5.4.1:** Effect of the control diet and the functional oil diet on end-point (d26/28) plasma total cholesterol concentrations for individual overweight women subjects (n=17).

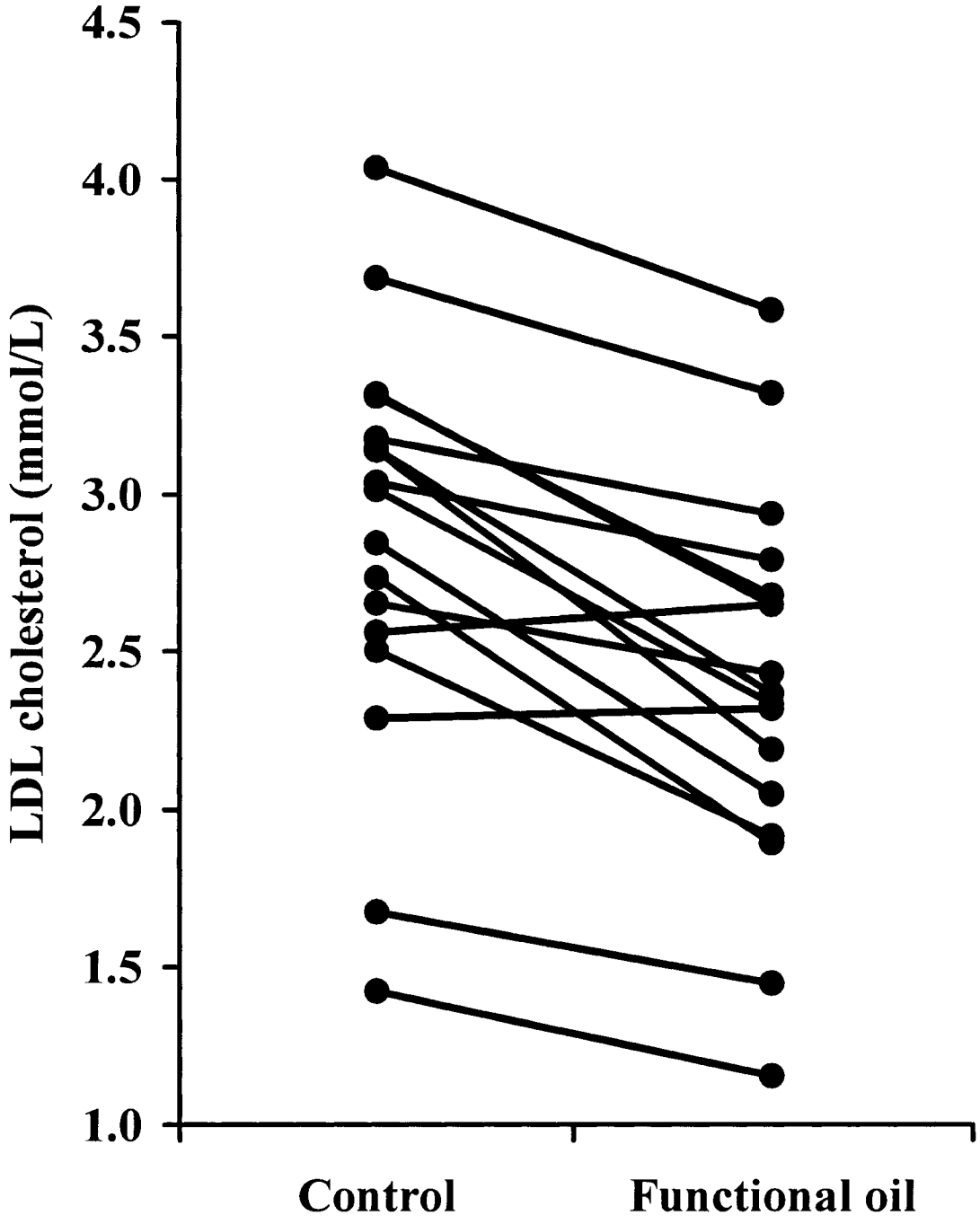


**Figure 5.4.2:** Effect of the control diet and the functional oil diet on end-point (d26/28) plasma LDL cholesterol concentrations for individual overweight women subjects (n=17).

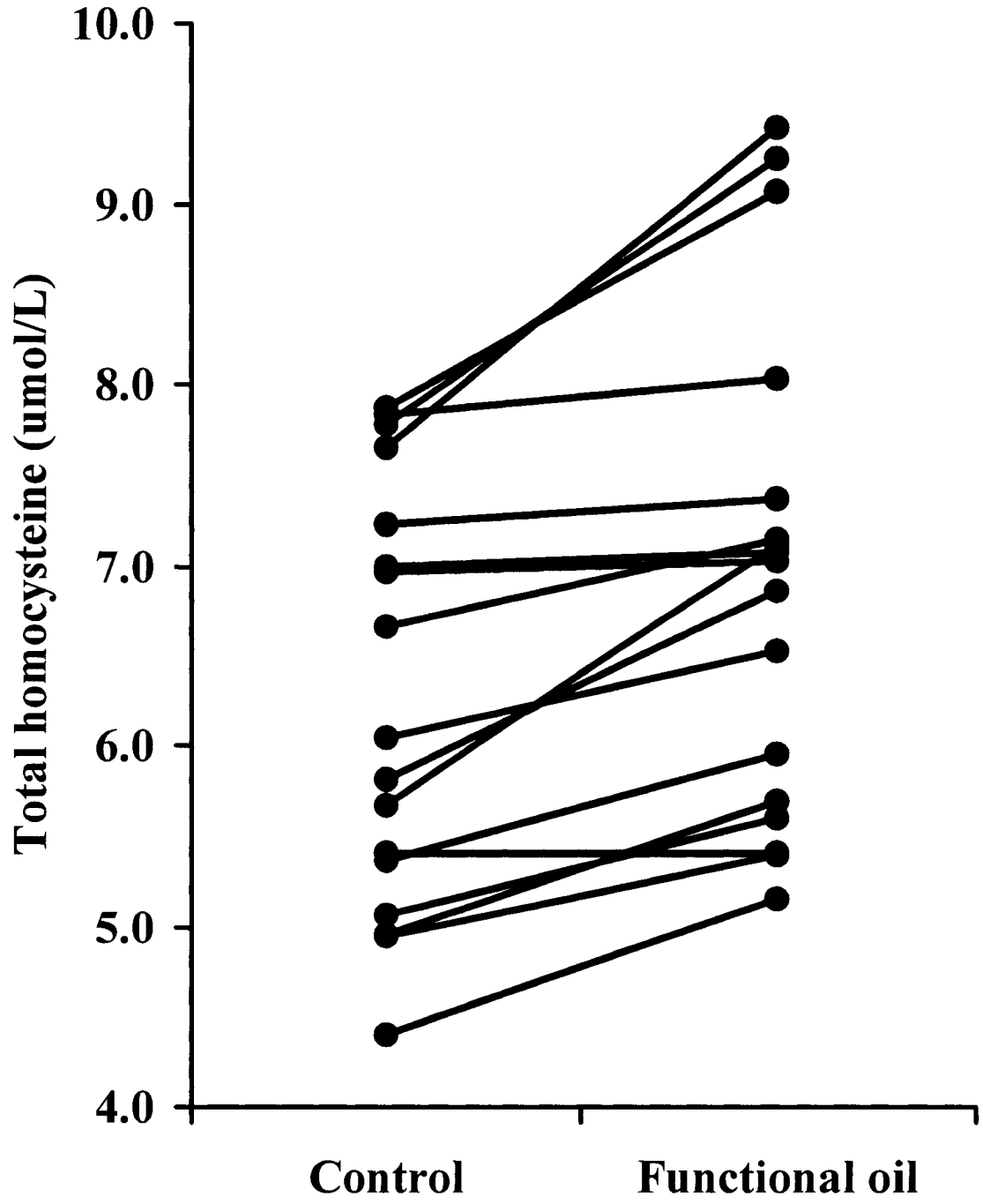


**Figure 5.4.3:** Effect of the control diet and the functional oil diet on end-point (d26/28) plasma total homocysteine concentrations for individual overweight women subjects (n=17).

## 5.5. Discussion

The present results demonstrate that consumption of a combination of MCT, phytosterols and n-3 PUFA in a controlled diet for 27 days substantially lowers total plasma and LDL cholesterol, but does not affect circulating TAG concentrations in healthy overweight women. Plasma total Hcy concentration was higher following the FctO vs C diet, although values remained within normal ranges. FctO supplementation increased the antioxidant glutathione.

A rigorously controlled inpatient setting and crossover design were used for this study. Subjects slept and resided exclusively at the CNRU and consumed precisely controlled diets under supervision, thereby assuring compliance to the dietary regimen. Furthermore, level of physical activity and menstrual cycle phase were controlled for in this feeding trial. Palatability of the FctO was excellent according to subjects, and diets were well tolerated, except for minor digestive discomforts during the initial adaptation period. Step-wise increments in the proportion of MCT could effectively resolve this difficulty, as suggested by Asakura et al (1999). Beef tallow in the C diet was selected to parallel the high degree of saturation of the FctO, while being free of MCT. C feeding did not alter any plasma lipid parameters, thus being an appropriate control from that perspective. Nonetheless, variations in red blood cell fatty acid composition within the C phase provide evidence that this diet did not perfectly parallel our subjects' habitual fat intake. In particular, decreases in tissue concentration of 14:0 and 16:0 following C feeding are indicative of the high saturated fat content in the usual diets of participants. In addition, beef tallow is derived from animal sources, and thus contains cholesterol. Hence, the C diet provided an extra 84mg/d of cholesterol on average compared to the FctO diet, and this may have acted to confound plasma cholesterol concentrations.

However, dietary cholesterol is recognized to have a minor impact on circulating cholesterol, in contrast to dietary fatty acid type and endogenous cholesterol (Jones and Papamandjaris, 2001).

Lower plasma LDL and total cholesterol, with mean endpoint differences of 16.4% and 9.0% respectively, were achieved when women in the present study substituted the FctO for the C fat. Compared to subjects' habitual intakes, LDL and total cholesterol were reduced by 10.4% and 4.8%, respectively, following consumption of the FctO diet. This extent of cholesterol lowering correlates well with those seen in other plant sterol supplementation trials (Miettinen et al, 1995; Pelletier et al, 1995; Jones et al, 1999; Hendriks et al, 1999; Jones et al, 2000; Tammi et al, 2000). When hypercholesterolemic subjects were fed tall oil phytosterols as part of a fixed-food and supervised diet for 30 days, total and LDL cholesterol were lower, by 9.1% and 15.5% respectively, in comparison to placebo (Jones et al, 1999). Although the present study design does not allow for attribution of specific dietary ingredients to changes in blood lipid parameters, the favourable influence on cholesterol metabolism was likely mediated by the presence of phytosterols, which are established cholesterol-lowering agents. It is also possible that flaxseed oil may have acted synergistically with phytosterols in lowering plasma cholesterol, thereby masking any hypercholesterolemic effect of MCT. Since consistently lower endpoint total and LDL cholesterol were observed for FctO, the beneficial effect is clinically important. Favourable increases in the protective HDL:LDL and HDL:total cholesterol ratios by 19.5% and 9.4%, respectively, provide further evidence that consumption of the FctO diet leads to substantial improvement to the CVD risk profile.

A major concern with utilization of MCT in an overweight population is the anticipated increase in plasma TAG concentrations. Flaxseed oil provides several advantages over fish oil as a source of n-3 PUFA. It is tasteless, odourless and does not require encapsulation for stability, hence allowing blinding of subjects and incorporation to a great variety of foods. A non-significant 4.1% decline in circulating TAG was observed with consumption of the present precisely controlled, alcohol-restricted, moderately high fat C diet, which provided isoenergetic meals. FctO feeding in these normotriglyceridemic overweight women did not significantly increase TAG concentrations. This may be the consequence of ALA in flaxseed oil preventing MCT-driven rise in TAG, or alternatively, the lack of influence of both ALA and MCT on TAG metabolism. Unchanged plasma TAG concentrations have indeed been reported following MCT (Wardlaw et al, 1995; Cater et al, 1997; Temme et al, 1997; Tsai et al, 1999; Asakura et al, 2000), and flaxseed oil (Cunnane et al, 1993; Layne et al, 1996; Goh et al, 1997; Pang et al, 1998) supplementation. However, TAG were reduced at the high flaxseed oil intake of 60mL per day for 2 weeks (Singer et al, 1986). With background diets low in total and saturated fat, lower ALA intakes were needed to effect TAG lowering (Indu and Ghafoorunissa, 1992). Increase in tissue EPA, but not DHA, on the FctO diet indicates ALA conversion to the long chain n-3 EPA, although the dose administered may have been insufficient for conversion to DHA. Indeed, conversion to DHA in vivo seems to be restricted (Emken et al, 1994). Flaxseed oil feeding has been reported to increase EPA only (Singer et al, 1986; Mantzioris et al, 1994; Goh et al, 1997), although small changes in DHA have also been observed (Layne et al, 1996). Nevertheless, EPA and DHA should have similar TAG-suppressing abilities (Grimsgaard et al, 1997), in circulation at least. Correlation analyses in this study do not support an

association between increases in tissue EPA, and declines in TAG. Feeding trials in hypertriglyceridemic individuals may be needed to ensure FctO safety for the general population, although MCT consumption in this subgroup has not shown detrimental effects (Asakura et al, 2000).

The significant difference in TAG between d26 and d28 of the FctO diet could be the consequence of the different meals consumed the day before each blood draw (roasted chicken on d25 and spaghetti on d27, Table 5.3.1), combined to the fact that all subjects consumed the same menu on these days, thus reducing inter-subject variability. This difference would then reflect the influence of different meals on fasting TAG concentrations, rather than the effect of treatment fat or variation in the analysis procedure, given the low coefficient of variation for TAG measurements (3.1%). Greater agreement observed between d26 and d28 values for cholesterol reflects the lesser influence of a single meal on these parameters.

Lack of weight maintenance on both dietary phases may be due to distribution of energy intake throughout the day, high fibre content of the basal diet, or alternatively, inadequate energy intake. One week of body weight monitoring may not have been sufficient to determine the appropriate weight-maintaining level of energy intake. In that respect, measurement of energy expenditure could provide a more accurate indication of individual energy needs. Comparable body weight reductions on both phases were assumed to have influenced blood lipids in a similar manner. The greater extent of fat absorption observed on the FctO vs C diet may be explained by the easier and faster digestion and absorption of MCT vs LCT, as confirmed by MCT use in malabsorption disorders (Bach et al, 1996). Improved MCT absorption, combined with equal fat intake, may have compensated for the increased energy expenditure of MCT, and thus prevented

a greater reduction in body weight following consumption of MCT, as compared to LCT. Differences between MCT and LCT absorption should be corrected for in future energy balance studies, although precise determination of total fecal fatty acids is complicated by the presence of bacterially-derived fat metabolites.

A reduction in plasma total Hcy was anticipated since the basal diet was high in folate, vitamin $B_{12}$, and vitamin $B_6$ (respective mean daily intakes: 409μg, 4.29μg and 2.67mg). Therefore, higher Hcy endpoints following FctO vs C supplementations were unexpected. Total Hcy concentrations remained within normal ranges in these subjects with low baseline values (range: 4.32-8.75μmol/L). However, the 10.8% difference in endpoint Hcy across diets may be of clinical concern for individuals with moderately elevated concentrations. In this study, the change in circulating Hcy was in opposite direction to that of cholesterol, conflicting with reports establishing a positive association between those two parameters (McCully et al, 1990; Stampfer et al, 1992; Anderson et al, 2000; Qujeq et al, 2001). The present increase in Hcy following dietary fat modulation in humans is unprecedented. To our knowledge, the effect of MCT or phytosterols on circulating total Hcy has not been investigated thus far, even though Hcy is recognized as a risk factor for CVD. Fish oil supplementation for 4 weeks decreased Hcy concentrations by 36-48% when compared to olive oil (Olszewski and McCully, 1993), although this effect has not been confirmed by other researchers (Grundt et al, 1999).

The MCT-derived increase in fat oxidation, energy expenditure and oxygen consumption may have generated oxidative by-products and thus reduced the capacity for Hcy clearance. This is consistent with increased production of oxygen radicals when stimulated leukocytes are incubated with a mixture of MCT and LCT, compared to LCT alone (Kruimel et al, 2000). The cofactor tetrahydrofolate, essential for recycling of Hcy

back to methionine, seems to be highly sensitive to oxidation, and it has been suggested that the hyperhomocysteinemia observed in CVD may be the result of oxidative depletion of folate, rather than insufficient dietary intake (Fuchs et al, 2001). In fact, it has been demonstrated that the folate-dependant recycling of Hcy is inhibited in the presence of oxidants, and under these conditions, the flux of Hcy through the transsulfuration pathway is increased, leading to downstream synthesis of glutathione (Mosharov et al, 2000). This regulatory mechanism is thought to be an adaptive response to raise levels of the antioxidant glutathione when oxidative stress is increased. Under these conditions, the decrease in methionine synthase and increase in methionine adenosyl transferase would then result in higher Hcy concentrations, which would further push the steady-state equilibrium towards greater glutathione synthesis (Mosharov et al, 2000). Therefore, favourable increase in the antioxidant glutathione observed on d28 of the FctO diet may be the result of an adaptative response to increased oxidative stress generated by MCT, with the metabolic consequence of increasing Hcy concentrations. Testing the FctO components separately for effects on Hcy metabolism may allow further elucidation into the mechanisms involved in dietary modulation of this aminothiol, and in its involvement with the development of atherosclerosis.

In conclusion, MCT administered with phytosterols and n-3 PUFA result in an overall positive influence on the CVD risk profile of healthy overweight women. This FctO therefore deserves further investigation for its possible role in weight management, although no difference in weight loss was observed between FctO and C diets in this study. Identification of dietary ingredients responsible for the observed higher Hcy concentrations with FctO may help to understand the role of Hcy in CVD.

# 6. FINAL CONCLUSION

## 6.1. Summary of Results

Supplementing diets with a FctO composed of MCT, phytosterols and n-3 PUFA for 27 days reduced plasma total cholesterol by 4.8% and LDL cholesterol by 10.4% relative to baseline. Plasma total and LDL cholesterol levels were lower on FctO vs C, with mean endpoint differences of 9.0% and 16.4 %, respectively. Dietary treatment did not affect HDL cholesterol or TAG in plasma. The protective ratios of HDL:total and HDL:LDL cholesterol were improved by 9% and 20% relative to baseline on FctO. Total Hcy was reduced on the C phase, but remained unchanged on the FctO phase, thus 11.4% higher endpoint values resulted from FctO vs C diet supplementation. Glutathione was increased at the end of the FctO phase compared to baseline. Consumption of the FctO diet increased tissue concentrations of 18:3n-3 and EPA, but not DHA, confirming the restricted conversion of ALA to DHA in vivo. Higher tissue 16:0 levels on the FctO diet, as compared to the C diet, supports de novo FA synthesis following MCT feeding. No difference in weight loss was observed between FctO and C diets. Higher fecal FA excretion on the FctO diet, as compared to the C diet, is consistent with the notion of a more rapid and complete absorption of MCT vs LCT. Lower fat absorption may explain the lack of preferential weight loss on the FctO diet. In conclusion, consumption of a controlled diet supplemented with the present FctO improves the overall cardiovascular risk profile of overweight women.

## 6.2. Future Research

Demonstration that the present FctO composed of MCT, phytosterols and n-3 PUFA, improves the CVD risk profile in a population of normo- to moderately

hyperlipidemic overweight women, warrants further research on the possible role of MCT in weight management. Additional investigations with longer feeding trials, where the increased absorption of MCT relative to LCT is adjusted for, should be considered. Such studies may allow the discernment of preferential weight loss with MCT vs LCT, when energy intake is fixed, such as provided by the present trial. Determination of the appropriate level of energy intake necessary for weight maintenance may require body weight monitoring for more than 1 week, or alternatively, the measurement of energy expenditure in individual subjects using indirect calorimetry.

In order to ensure the safety and appropriateness of this FctO for consumption by the general population, further testing on hypertriglyceridemic subjects should be undertaken, since such individuals may respond differently to MCT feeding. The usefulness of flaxseed oil in the present FctO, given the observed absence of TAG reduction, remains to be confirmed. Due to restricted conversion of ALA to EPA and DHA in tissue, the low levels of long chain n-3 PUFA synthesized following flaxseed oil consumption may be insufficient to modulate circulating TAG levels (Cunnane et al, 1993; Layne et al, 1996; Goh et al, 1997; Pang et al, 1998). Marine n-3 PUFA may be more efficacious in lowering plasma TAG; however, they may not be easily incorporated into functional foods due to taste, odour and stability concerns. Flaxseed oil can be consumed throughout the day since it is tasteless and odourless, and can be blended into oils and foods since it does not require encapsulation for stability. In addition, flaxseed oil contains sufficient vitamin E to counteract possible increases in oxidative stress following consumption of unsaturated n-3 FA (Simopoulos, 1999). In contrast, fish oil contains much less vitamin E and therefore may promote oxidative damage in the event of inadequate antioxidant intake. Examination of the effect of MCT, combined with

phytosterols alone, on the TAG metabolism of hyperlipidemic subjects would allow to determine whether the n-3 PUFA fraction of this experimental oil is beneficial. In the event that the mixture would not be detrimental to TAG metabolism, absence of flaxseed oil could allow incorporation of the modified FctO oil into a greater variety of functional foods, by permitting heating in the cooking process without concern to lipid peroxidation. However, it may still be worthwhile to retain flaxseed oil as part of the FctO since intake of the essential ALA has been found inversely associated with risk of heart disease, and ALA consumption is known to improve many other parameters related to CVD, such as thrombogenesis, ventricular fibrillation, platelet clotting, and blood pressure (Simopoulos, 2000).

Demonstration for the first time that phytosterols, when administered in a FctO mainly composed of MCT, are efficacious in lowering total and LDL cholesterol confirms the beneficial effect of the present FctO to help prevent CVD. Although the safety of phytosterols has already been evaluated, their combination with MCT may exacerbate the block of absorption of fat-soluble vitamins, since micellar formation is reduced with MCT. Therefore, variations in the levels of these nutrients in plasma and in stores should be measured following FctO supplementation in order to determine whether addition of certain fat-soluble vitamins to the FctO is needed.

The palatability of beef tallow in the C diet seemed to have been problematic in this study since it resulted in the withdrawal of two subjects. Furthermore, beef tallow may not appropriately reflect habitual fat intake in Western societies, given the variations observed in tissue FA on the C diet. Future studies may consider a more suitable control fat that would correspond to the current recommendation, such as olive oil, or alternatively, a mixture of oils that would match the relative proportion of FA usually

consumed in the general population. However, such alternatives as controls would provide a lower proportion of saturated fat and a higher unsaturated:saturated FA ratio, in comparison to the FctO, and these may act as confounders to lipid and energy metabolism.

Of particular interest are higher plasma total Hcy endpoints following FctO vs C feeding, given that basal diets were identical except for treatment fat. Although total plasma Hcy levels remained within the normal range in subjects with low circulating baseline values, the difference observed between the two diets may lead to detrimental consequences in individuals with moderately elevated Hcy levels and prone to heart disease. Few studies have examined the effect of dietary fat modification on Hcy metabolism. The observed higher levels of total Hcy in this study following changes to the FA and sterol content is interesting and should be further investigated in order to understand the dietary modulation of this aminothiol and its involvement with the development of CVD. A plausible hypothesis involves the MCT-derived increase in fat oxidation, energy expenditure and oxygen consumption, which may have increased oxidative stress. This is consistent with the oxidative theory of the development of atherosclerosis, and the fact that elevated plasma Hcy levels are found in heavy smokers (Hankley and Eikelboom, 1999). Higher production of oxygen radicals resulted when stimulated leukocytes were incubated with a mixture of MCT and LCT, compared to LCT alone (Kruimel et al, 2000). The observed favourable increase in the antioxidant glutathione on d28 of the FctO diet may result from an adaptative response to increased oxidative stress (Mosharov et al, 2000) generated by MCT, with the metabolic consequence of increasing Hcy concentrations. The presence of highly unsaturated n-3 FA may also have increased oxidative stress, although flaxseed oil contains plenty of

antioxidants, and fish oil has been previously reported to decrease Hcy levels (Olszewski and McCully, 1993). Examining the separate effects of MCT, phytosterols and n-3 PUFA on Hcy levels and on different indices of oxidative stress, including lipid peroxides, may provide some insight into these hypotheses.

## 6.3. Significance

This study demonstrates that consumption of a FctO composed of MCT, phytosterols and n-3 PUFA improves the overall cardiovascular risk profile in overweight women. This FctO therefore deserves further investigation for its possible role in promoting body weight loss and in reducing the risk of obesity-related conditions, through a negative impact on energy balance. The net energy deficit that may be generated by consumption of this FctO when substituted for a typical fat could assist obese individuals in losing body weight and also provide an opportunity for normal-weight individuals to prevent weight gain over the long term. Therefore, incorporation of this FctO into the diet of individuals at increased risk of developing CVD or obesity could have a positive impact on the incidence of these widespread chronic conditions in Western societies.

## BIBLIOGRAPHY

Abbey M, Clifton P, Kestin M, Belling B, Nestel P. Effect of fish oil on lipoproteins, lecithin:cholesterol acyltransferase, and lipid transfer protein activity in humans. Arteriosclerosis 1990;10:85-94.

Agarwal S, Rao AV. Tomato lycopene and its role in human health and chronic diseases. Can Med Ass J 2000;163:739-44.

Al Makdessi S, Andrieu JL, Bacconin A, Fugier JC, Herilier H, Faucon G. Assay of lipids in dog myocardium using capillary gas chromatography and derivatization with boron trifluoride and methanol. J Chromatogr 1985;339:25-34.

Anderson JL, Muhlestein JB, Horne BD et al. Plasma homocysteine predicts mortality independently of traditional risk factors and C-reactive protein in patients with angiographically defined coronary artery disease. Circulation 2000;102:1227-32.

Asakura L, Lottenberg AMP, Neves MQTS et al. Dietary medium-chain triacylglycerol prevents the postprandial rise of plasma triacylglycerols but induces hypercholesterolemia in primary hypertriglyceridemic subjects. Am J Clin Nutr 2000;71:701-5.

Ayesh R, Weststrate JA, Drewitt PN, Hepburn PA. Safety evaluation of phytosterol esters. Part 5. Faecal short-chain fatty acid and microflora content, faecal bacterial enzyme activity and serum female sex hormones in healthy normolipidaemic volunteers consuming a controlled diet either with or without a phytosterol ester-enriched margarine. Food Chem Toxicol 1999;37:1127-38.

Bach AC, Babayan VK. Medium-chain triglycerides: An update. Am J Clin Nutr 1982;36:950-62.

Bach AC, Ingenbleek Y, Frey A. The usefulness of dietary medium-chain triglycerides in body weight control: Fact or fancy? J Lipid Res 1996;37:708-26.

Bang HO, Dyerberg J. Lipid metabolism and ischemic heart disease in Greenland Eskimos. In: Advances in nutrition research. Draper H, ed. New York: Plenum Press, 1980:1-22.

Beveridge JMR, Connell WF, Haust HL, Mayer GA. Dietary cholesterol and plasma cholesterol levels in man. Can J Biochem Physiol 1959;37:575-82.

Binnert C, Pachiaudi C, Beylot M et al. Influence of human obesity on the metabolic fate of dietary long- and medium-chain triacylglycerols. Am J Clin Nutr 1998;67:595-601.

Blair SN, Capuzzi DM, Gottlieb SO, Nguyen T, Morgan JM, Cater NB. Incremental reduction of serum total cholesterol and low-density lipoprotein cholesterol with the addition of plant stanol ester-containing spread to statin therapy. Am J Cardiol 2000;86:46-52.

Canada Gazette Directorate, Public Works and Government Services Canada: Ottawa (ON). Regulations amending the food and drug regulations (nutrition labelling, nutrition claims and health claims). Canada Gazette: Part I, June 16, 2001:135(24). Available: www.hc-sc.gc.ca/food-aliment/english/publications/acts_and_regulations/part_1/1172_index.html.

Carnielli VP, Sulkers EJ, Moretti KC et al. Conversion of octanoic acid into long-chain saturated fatty acids in premature infants fed a formula containing medium-chain triglycerides. Metabolism Clin Exper 1994;43:1287-92.

Cater NB, Heller HJ, Denke MA. Comparison of the effects of medium-chain triacylglycerols, palm oil, and high oleic acid sunflower oil on plasma triacylglycerol fatty acids and lipid and lipoprotein concentrations in humans. Am J Clin Nutr 1997;65:41-5.

Chan JK, Bruce VM, McDonald BE. Dietary alpha-linolenic acid is as effective as oleic acid and linoleic acid in lowering blood cholesterol in normolipidemic men. Am J Clin Nutr 1991;53:1230-4.

Connor WE. Importance of n-3 fatty acids in health and disease. Am J Clin Nutr 2000;171:171S-5S.

Craig WJ. Phytochemicals: Guardians of our health. J Am Diet Assoc 1997;97:S199-204.

Crozier GL. Medium-chain triglyceride feeding over the long term: The metabolic fate of [$^{14}$C]Octanoate and [$^{14}$C]Oleate in isolated rat hepatocytes. J Nutr 1988;118:297-304.

Cunnane SC, Ganguli S, Menard C et al. High alpha-linolenic acid flaxseed (Linum usitatissimum): Some nutritional properties in humans. B J Nutr 1993;69:443-53.

Denke MA. Lack of efficacy of low-dose sitostanol therapy as an adjunct to a cholesterol-lowering diet in men with moderate hypercholesterolemia. Am J Clin Nutr 1995;61:392-6.

Dulloo AG, Fathi M, Mensi N, Girardier L. Twenty-four-hour energy expenditure and urinary cathecolmines of humans consuming low-to-moderate amounts of medium-chain triglycerides: A dose-response study in a human respiratory chamber. Eur J Clin Nutr 1996;50:152-8.

Durand P, Fortin LJ, Lussier-Cacan S, Davignon J, Blache D. Hyperhomocysteinemia induced by folic acid deficiency and methionine load – applications of a modified HPLC method. Clin Chim Acta 1996;252:83-93.

Emken EA, Adolf RO, Gulley RM. Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young adult males. Biochim Biophys Acta 1994;1213:277-88.

Fokkema MR, Brouwer DAJ, Hasperhoven MB, Martini IA, Muskiet FAJ. Short-term supplementation of low-dose gamma–linolenic acid (GLA), alpha-linolenic acid (ALA), or GLA plus ALA does not augment LCP omega-3 status of Dutch vegans to an appreciable extent. Prostaglandins Leukot Essent Fatty Acids 2000;63:287-92.

Folch J, Lees M, Sloan S. A simple method for the isolation and purification of the total lipids from animal tissues. J Biol Chem 1957;226:497-509.

Food and Drug Administration, US Department of Health and Human Services. Regulatory impact analysis of the final rules to amend the food labeling regulations. Federal Register 1993;58:2927-41.

Food and Drug Administration Food Labeling, U.S. Department of Health and Human Services. Health Claims; Soluble fiber from whole oats and risk of coronary heart disease; Final rule. Federal Register: March 31, 1997;62(61).

Food and Drug Administration, U.S. Department of Health and Human Services. Food Labeling: Health Claims; Calcium and osteoporosis. Codes of Federal Regulations: April 1, 1999;21(2): parts 100-69.

Food and Drug Administration, U.S. Department of Health and Human Services. FDA authorizes new coronary heart disease health claim for plant sterol and plant stanol esters. Federal Register: September 8, 2000;65(175).

Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.

Fuchs D, Jaeger M, Widner B, Wirleitner B, Artner-Dworzak E, Leblhuber F. Is hyperhomocysteinemia due to the oxidative depletion of folate rather than to insufficient dietary intake? Clin Chem Lab Med 2001;39:691-4.

Geliebter A, Torbay N, Bracco EB, Hashim SA, Van Italie TB. Overfeeding with medium-chain triglyceride diet results in diminished deposition of fat. Am J Clin Nutr 1983;37:1-4.

Giovannini M, Agostoni C, Salari PC. The role of lipids in nutrition during the first months of life. J Int Med Res 1991;19:351-62.

Goh YK, Jumpsen JA, Clandinin MT. Effect of omega 3 fatty acid on plasma lipids, cholesterol and lipoprotein fatty acid content in NIDDM patients. Diabetologia 1997;40:45-52.

Goldfarb: Consultants for Health Canada, Nutrition Evaluation Division Food Directorate. Health Claims Focus Testing: October 2000. October 2000. Available: www.hc-sc.gc.ca/food-aliment/english/subjects/health_claims/hc_focus_testing.html.

Grimsgaard S, Bonaa KH, Hansen JB, Nordoy A. Highly purified eicosapentaenoic acid and docosahexeanoic acid in human have similar triacylglycerol-lowering effects but divergent effects on serum fatty acids. Am J Clin Nutr 1997;66:649-59.

Grundt H, Nilsen DWT, Hetland O, Mansoor MA, Aarsland T, Woie L. Atherothrombogenic risk modulation by n-3 fatty acids was not associated with changes in homocysteine in subjects with combined hyperlipidaemia. Thromb Heamost 1999;81:561-5.

Gylling H, Miettinen TA. Cholesterol reduction by different plant stanol mixtures and with variable fat intake. Metabolism 1999;48:575-80.

Hainer V, Kunesova M, Stich V, Zak A, Parizkova J. [The role of oils containing triacylglycerols and medium-chain fatty acids in the dietary treatment of obesity. The effect on resting energy expenditure and serum lipids]. [Abstract in Czech] Cas Lek Cesk 1994;133:373-5.

Hallikainen MA, Sarkkinen ES, Gylling H, Erkkila AT, Uusitupa MIJ. Comparison of the effects of plants sterol ester and plant stanol ester-enriched margarines in lowering serum cholesterol concentrations in hypercholesterolaemic subjects on a low-fat diet. Eur J Clin Nutr 2000;54:715-25.

Hankley GJ, Eikelboom JW. Homocysteine and vascular disease. The Lancet 1999;354:407-13.

Harris WS. n-3 Fatty acids and serum lipoproteins: Human studies. Am J Clin Nutr 1997;65: S1645-54.

Health Canada. Recommended nutrient intakes for Canadians. Department of National Health and Welfare: Ottawa (ON). 1990.

Health Canada, Therapeutic Products Programme and the Food Directorate of the Health Protection Branch: Ottawa (ON). Nutraceuticals/functional foods and health claims on foods policy: Policy paper. November 2, 1998. Available: www.hc-sc.gc.ca/hpb-dgps/ therapeut/zfiles/english/ffn/nutra_pol_e.pdf.

Health Canada, Food Directorate Branch, Bureau of Nutritional Sciences: Ottawa (ON). Standards of evidence for evaluating foods with health claims: A proposed framework. June 2000. Available: www.hc-sc.gc.ca/food-aliment/english/subjects/health_claims/ Consultation_doc_en.pdf.

Health Canada Food Directorate Branch, Bureau of Nutritional Sciences: Ottawa (ON). Consultation document on generic health claims. August 2000. Available: www.hc-sc.gc.ca/food-aliment/english/subjects/health_claims/consultation_doc_ gen.html.

Health Canada, Food Directorate Branch, Bureau of Nutritional Sciences: Ottawa (ON). Chronology of events: Policy development process. Available: www.hc-sc.gc.ca/food-aliment/english/subjects/health_claims/chronology_of_events_.html.

Health Canada: Ottawa (ON). Advisory: Health Canada advises that Becel™ Pro-activ™ not approved for sale. October 3, 2001. Available: www.hc-sc.gc.ca/english/archives/warnings/2001/ 2001_106e.htm.

Heinemann T, Leiss O, von Bergmann K. Effect of low-dose sitostanol on serum cholesterol in patients with hypercholesterolemia. Atherosclerosis 1986;61:219-23.

Hendriks HFJ, Weststrate JA, van Vliet T, Meijer GW. Spreads enriched with three different levels of vegetable oil sterols and the degree of cholesterol lowering in normocholesterolaemic and mildly hypercholesterolaemic subjects. Eur J Clin Nutr 1999;53:319-27.

Hill JO, Peters JC, Swift LL et al. Changes in blood lipids during six days of overfeeding with medium or long chain triglycerides. J Lipid Res 1990;31:407-16.

Hill JO, Peters JC, Yang D et al. Thermogenesis in humans during overfeeding with medium-chain triglycerides. Metabolism 1989;38:641-8.

Hornstra G, Barth CA, Galli C et al. Functional food science and the cardiovascular system. Br J Nutr 1998;80:S113-46.

Indu M, Ghafoorunissa. n-3 Fatty acids in Indian diets-comparison of the effects of precursor (alpha-linolenic acid) vs product (long chain n-3 polyunsaturated fatty acids). Nutr Res 1992;12:569-82.

Jacobsen DW. Homocysteine and vitamins in cardiovascular disease. Clin Chem 1998;44:1833-43.

Johnson RC, Young SK, Cotter R, Lin L, Rowe WB. Medium-chain-triglyceride lipid emulsion: Metabolism and tissue distribution. Am J Clin Nutr 1990;52:502-8.

Jones PJ, MacDougall DE, Ntanios F, Vanstone CA. Dietary phytosterols as cholesterol-lowering agents in humans. Can J Physiol Pharmacol 1997;75:217-27.

Jones PJ, Howell T, MacDougall DE, Feng JY, Parsons W. Short-term administration of tall oil phytosterols improves plasma lipid profiles in subjects with different cholesterol levels. Metabolism 1998;47:751-6.

Jones PJH, Ntanios FY, Raeini-Sarjaz M, Vanstone CA. Cholesterol-lowering efficacy of a sitostanol-containing phytosterol mixture with a prudent diet in hyperlipidemic men. Am J Clin Nutr 1999;69:1144-50.

Jones PJ, Raeini-Sarjaz M, Ntanios FY, Vanstone CA, Feng JY, Parsons WE. Modulation of plasma lipid levels and cholesterol kinetics by phytosterol versus phytostanol esters. J Lipid Res 2000;41:697-705.

Jones PJH, Papamandjaris AA. Lipids: Cellular metabolism. In: Present knowledge in nutrition, 8th ed. Bowman BA, Russell RM, ed. Washington, DC: ILSI Press, 2001:104-14.

Katan MB, Zock PL, Mensink RP. Dietary oils, serum lipoproteins, and coronary heart disease. Am J Clin Nutr 1995; 61: S1368-73.

Kris-Etherton PM, Yu S. Individual fatty acid effects on plasma lipids and lipoproteins: Human studies. Am J Clin Nutr 1997;65: S1628-44.

Kromhout D, Bosschieter EB, de Lezenne Coulander C. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. N Engl J Med 1985;312:1205-9.

Kruimel JW, Naber AH, Curfs JH, Wenker MA, Jansen JB. With medium-chain triglycerides, higher and faster oxygen radical production by stimulated polymorphonuclear leukocytes occurs. J Parenter Enteral Nutr 2000;24:107-12.

Lavau MM, Hashim SA. Effect of medium chain triglyceride on lipogenesis and body fat in the rat. J Nutr 1978;108:613-20.

Layne KS, Goh YK, Jumpsen JA, Ryan EA, Chow P, Clandinin MT. Normal subjects consuming physiological levels of 18:3(n-3) and 20:5(n-3) from flaxseed or fish oils have characteristic differences in plasma lipid and lipoprotein fatty acid levels. J Nutr 1996;126:2130-40.

Lees AM, Mok HYI, Lees RS, McCluskey MA, Grundy SM. Plant sterols as cholesterol-lowering agents: Clinical trials in patients with hypercholesterolemia and studies of sterol balance. Atherosclerosis 1977;28:325-38.

Mantzioris E, James MJ, Gibson RA, Cleland LG. Dietary substitution with an alpha-linolenic acid-rich vegetable oil increases eicosapentanoic acid concentrations in tissues. Am J Clin Nutr 1994;59:1304-9.

Mascioli EA, Randall S, Porter KA et al. Thermogenesis from intravenous medium-chain triglycerides. J Parenter Enteral Nutr 1991;15:27-31.

Matsuo T, Matsuo M, Taguchi N, Takeuchi H. The thermic effect is greater for structured medium- versus long-chain triacylglycerols in healthy young women. Metabolism 2001;50:125-30.

McCully KS, Olszewski AJ, Vezeridis MP. Homocysteine and lipid metabolism in atherogenesis: Effect of the homocysteine thiolactonyl derivatives, thioretinaco and thioretinamide. Atherosclerosis 1990;83:197-206.

McGandy RB, Hegsted DM, Myers ML. Use of semisynthetic fats in determining effects of specific dietary fatty acids on serum lipids in man. Am J Clin Nutr 1970;23:1288-98.

Miettinen TA, Puska P, Gylling H, Vanhanen H, Vartiainen E. Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. N Engl J Med 1995;333:1308-12.

Mifflin MD, St Jeor ST, Hill LA, Scott BJ, Daugherty SA, Koh YD. A new predictive equation for resting energy expenditure in healthy individuals. Am J Clin Nutr 1990;51:241-7.

Mosharov E, Cranford MR, Banerjee R. The quantitatively important relationship between homocysteine metabolism and glutathione synthesis by the transsulfuration pathway and its regulation by redox changes. Biochemistry 2000;39:13005-11.

National Institutes of Health, National Heart, Lung, and Blood Institute, US Department of Health and Human Services. Clinical guidelines on the identification, evaluation, and the treatment of overweight and obesity in adults: The evidence report. Bethesda, MD:1998. [NIH Publication No. 98-4083.]

Nygard O, Vollset SE, Refsum H et al. Total homocysteine and cardiovascular risk profile. JAMA 1995;274:1526-33.

Olszewski AJ, McCully KS. Homocysteine content of lipoproteins in hypercholesterolemia. Atherosclerosis 1991;88:61-8.

Olszewski AJ, McCully KS. Fish oil decreases serum homocysteine in hyperlipemic men. Coron Artery Dis 1993;4:53-60.

Olszewski AJ, Szostak WB, Bialkowska M, Rudnicki S, McCully KS. Reduction of plasma lipid and homocysteine levels by pyridoxine, folate, cobalamin, choline, riboflavin, and troxerutin in atherosclerosis. Atherosclerosis 1989;75:1-6.

Pang D, Allman-Farinelli MA, Wong T, Barnes R, Kingham KM. Replacement of linoleic acid with alpha-linolenic acid does not alter blood lipids in normolipidaemic men. Br J Nutr 1998;80:163-7.

Papamandjaris AA, MacDougall DE, Jones PJH. Medium chain fatty acid metabolism and energy expenditure: Obesity treatment implications. Life Sciences 1998;62:1203-15.

Pawlosky RJ, Hibbeln JR, Novotny JA, Salem N Jr. Physiological compartmental analysis of alpha-linolenic acid metabolism in adult humans. J Lipid Res 2001;42:1257-65.

Pelletier X, Belbraouet S, Mirabel D et al. A diet moderately enriched in phytosterols lowers plasma cholesterol concentrations in normocholesterolemic humans. Ann Nutr Metab 1995;39:291-5.

Plat J, Mensink RP. Vegetable oil based versus wood based stanol ester mixtures: Effects on serum lipids and hemostatic factors in non-hypercholesterolemic subjects. Atherosclerosis 2000;148:101-12.

Qujeq D, Omran TS, Hosini LH. Correlation between total homocysteine, low-density lipoprotein cholesterol and high density lipoprotein cholesterol in the serum of patients with myocardial infarction. Clin Biochem 2001;34:97-101.

Roche HM, Gibney MJ. Effect of long-chain n-3 polyunsaturated fatty acids on fasting and postprandial triacylglycerol metabolism. Am J Clin Nutr 2000;71: S232-7.

SAS Institute Inc. SAS online documentation, version 8. Cary, NC: SAS Institute Inc, 1999.

Scalfi L, Coltori A, Contaldo F. Postprandial thermogenesis in lean and obese subjects after meals supplemented with medium-chain and long-chain triglycerides. Am J Clin Nutr 1991;53:1130-3.

Seaton TB, Welle SL, Warenko MK, Campbell RG. Thermic effect of medium-chain and long-chain triglycerides in man. Am J Clin Nutr 1986;44:630-4.

Shils ME, Olson JA, Shike M, Ross AC, ed. Appendix A-11-e: Average daily energy requirement of adults whose occupational work is classified as light, moderate, or heavy, expressed as a multiple of basal metabolic rate. In: Modern nutrition in health and disease, 9th ed. Baltimore, MD: Williams & Wilkins, 1999.

Simon E, Fernandez-Quintela A, Del Puy Portillo M, Del Barrio AS. Effects of medium-chain fatty acids on body composition and protein metabolism in overweight rats. J Physiol Biochem 2000;56:337-46.

Simopoulos AP. Essential fatty acids in health and chronic disease. Am J Clin Nutr 1999;70: S560-9.

Singer P, Berger I, Wirth M, Godicke W, Jaeger W, Voigt S. Slow desaturation and elongation of linoleic and alpha-linolenic acids as a rationale of eicosapentanoic acid-rich diet to lower blood pressure and serum lipids in normal, hypertensive and hyperlipemic subjects. Prostaglandins Leukot Med 1986;24:173-93.

Stampfer MJ, Malinow MR, Willet WC et al. A prospective study of plasma homocysteine and risk of myocardial infarction in US physicians. JAMA 1992;268:877-81.

Statistics Canada and Health Canada, Heart and Stroke Foundation, Health Statistics, Division of Heart Disease and Stroke in Canada: Ottawa (ON), Canada: 1997.

Swift LL, Hill JO, Peters JC, Greene HL. Medium-chain fatty acids: Evidence for incorporation into chylomicron triglycerides in humans. Am J Clin Nutr 1990;52:834-6.

Swift LL, Hill JO, Peters JC, Green HL. Plasma lipids and lipoproteins during 6 d of maintenance feeding with long-chain, medium-chain, and mixed-chain triglycerides. Am J Clin Nutr 1992;56:881-6.

Tammi A, Ronnemaa T, Gylling H et al. Plant stanol ester margarine lowers serum total and low-density lipoprotein cholesterol concentrations of healthy children: The STRIP project. J Pediatr 2000;136:503-10.

Temme EHM, Mensink RP, Hornstra G. Effects of medium chain fatty acids (MCFA), myristic acid, and oleic acid on serum lipoproteins in healthy subjects. J Lipid Res 1997;38:1746-54.

Thambyrajah J, Townend JN. Homocysteine and atherothrombosis-mechanisms for injury. Eur Heart J 2000;21:967-74.

Tsai YH, Park S, Kovacic J, Snook JT. Mechanisms mediating lipoprotein responses to diets with medium-chain triglyceride and lauric acid. Lipids 1999;34:895-905.

Unilever Canada: Toronto (ON). Unilever Canada Stands Behind Safety of New Becel Pro.activ Margarine. October 4, 2001.Available: www.unilever.ca/news/PressReleases.

Unnevehr L, Ward MR, Hasler C. Regulating health claims on food products: The balance between consumer choice and consumer protection. Choices First Quarter 1998; 26-30.

Vanhanen HT, Blomqvist S, Ehnholm C et al. Serum cholesterol, cholesterol precursors, and plant sterols in hypercholesterolemic subjects with different apoE phenotypes during dietary sitostanol ester treatment. J Lipid Res 1993;34:1535-44.

Vermunt SH, Mensink RP, Simonis MM, Hornstra G. Effects of dietary alpha-linolenic acid on the conversion and oxidation of 13C-alpha-linolenic acid. Lipids 2000;35:137-42.

Waalkens-Berendsen DH, Wolterbeek APM, Wijnands MVW, Richold M, Hepburn PA. Safety evaluation of phytosterol esters. Part 3. Two-generation reproduction study in rats with phytosterol esters-a novel functional food. Food Chem Toxicol 1999;37:683-96.

Wardlaw GM, Snook JT, Park S et al. Relative effects on serum lipids and apolipoproteins of a caprenin-rich diet compared with diets rich in palm oil/palm-kernel or butter. Am J Clin Nutr 1995;61:535-42.

Warnick GR, Benderson J, Albers JJ. Dextran sulfate-$Mg^{2+}$ precipitation procedure for quantitation of high-density-lipoprotein cholesterol. Clin Chem 1982;28:1379-88.

Weinberger MH. Salt and blood pressure. Curr Opin Cardiol 2000;15:254-7.

Weststrate JA, Meijer GW. Plant sterol-enriched margarines and reduction of plasma total- and LDL-cholesterol concentrations in normocholesterolaemic and mildly hypercholesterolaemic subjects. Eur J Clin Nutr 1998;52:334-43.

Whittaker MH, Frankos VH, Wolterbeek APM, Waalkens-Berendsen DH. Two-generation reproductive toxicity study of plant stanol esters in rats. Regul Toxicol Pharmacol 1999;29:196-204.

White MD, Papamandjaris AA, Jones PJH. Enhanced postprandial energy expenditure with medium-chain fatty acid feeding is attenuated after 14 d in premenopausal women. Am J Clin Nutr 1999;69:883-9.

Yost TJ, Eckel RH. Hypocaloric feeding in obese women: Metabolic effects of medium-chain triglyceride substitution. Am J Clin Nutr 1989;49:326-30.

Zulli A, Buxton B, Doolan L, Liu JJ. Effect of homocysteine and cholesterol in raising plasma homocysteine, cholesterol and triglyceride levels. Life Sci 1998;62:2191-4.