GENETIC HETEROGENEITY IN THE IMPACT OF DAIRY PRODUCT CONSUMPTION ON CHOLESTEROL METABOLISM IN HUMANS

by

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ABSTRACT

With the increased marketing and popularity of a range of dairy products in recent years, research has become widespread concerning the influence of dairy on human health. It is also becoming evident that an individual's genetic make-up contributes to shaping their health responses to dietary intakes. This research was primarily designed to investigate the impact of genetic variability on responsiveness of cholesterol metabolism, a classic biomarker of cardiovascular health, to the recommended level of dairy consumption in Canada. A secondary objective was to assess the influence of dairy intake on systemic inflammation as an emerging risk factor for cardiovascular disease. In a multicentre, randomized, free-living crossover design, 124 healthy individuals consumed 3 servings/day of conventional low-fat and regular milk, yogurt, and cheese (DAIRY diet) or dairy-free control products (CONTROL diet), each for 28 days as part of a prudent background dietary protocol. At the end of the study, DAIRY was associated with increased plasma concentrations of two established fatty acid biomarkers of dairy fat, pentadecanoic acid (C15:0) and heptadecanoic acid (C17:0), as well as with small increases in serum total cholesterol (TC) and LDL-cholesterol (LDL-C) concentrations. Inter-individual variability in the cholesterol transport gene *ABCG5*, bile acid synthesis gene *CYP7A1*, and cholesterol synthesis gene *DHCR7* contributed to shaping the degree of TC and LDL-C responsiveness to DAIRY; with higher cholesterol concentrations observed among *ABCG5* rs6720173-G/G homozygotes, *CYP7A1* rs3808607-G allele carriers, and *DHCR7* rs760241-A allele carriers, relative to the C allele, T/T, and G/G carriers of these genes, respectively. Also, after DAIRY, the major allele T homozygosity of *CYP7A1* rs3808607 and the minor allele A of *DHCR7* rs760241 were associated with

reduced plasma [3,4]^{13}C cholesterol enrichment and deuterium incorporation, respectively, suggesting reduced cholesterol absorption and synthesis rates. DAIRY intake did not influence the inflammatory status. Overall, this research has provided evidence of a potential impact of the genomic architecture on responsiveness of cholesterol metabolism to dairy consumption. The novel findings are expected to advance knowledge of the inherited basis by which health biomarkers may be modified in response to whole foods, hence launching an important step towards an era of personalized nutrition.

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ABBREVIATIONS

7αHC	7-alpha-hydroxycholesterol
25(OH)D	25-hydroxyvitamin D
ABCA1	ATP-binding cassette, sub-family A1
ABCG5	ATP-binding cassette, sub-family G (WHITE), member 5
ABCG8	ATP-binding cassette, sub-family G (WHITE), member 8
ADIPOQ	Adiponectin
ANOVA	Analysis of variance
APOA	Apolipoprotein A
APOB	Apolipoprotein B
APOC3	Apolipoprotein C3
APOE	Apolipoprotein E
AUC	Area under the [3, 4]^{13}C cholesterol RBC enrichment curve
BMI	Body mass index
CCL2	Chemokine (C-C motif) ligand 2
CD36	Cluster of differentiation 36
CETP	Cholesteryl ester transfer protein
CHD	Coronary heart disease
CLA	Conjugated linoleic acid
CHO	Carbohydrate
CVD	Cardiovascular disease
CYP7A1	Cholesterol 7 alpha-hydroxylase
DHCR7	7-dehydrocholesterol reductase

DNA	Deoxyribonucleic acid
FA	Fatty acid
FABP2	Fatty acid binding protein 2
FFQ	Food frequency questionnaire
FRS	Framingham 10-year CHD risk score
FSH	Follicle-stimulating hormone
FSR	Fractional synthesis rate
G6PD	Glucose-6-phosphate dehydrogenase
GC	Gas chromatography
GC-FID	Gas chromatography - flame ionization detector
GC-MS	Gas chromatography - mass spectrometry
GWAS	Genome-wide association study
HDL-C	High-density lipoprotein cholesterol
HMGCR	3-hydroxy-3-methyl-glutaryl-coenzyme A reductase
HPLC	High performance liquid chromatography
hs-CRP	High-sensitivity C-reactive protein
IL	Interleukin
INAF	Institute of Nutrition and Functional Foods
IRMS	Isotope ratio mass spectrometry
LDL-C	Low-density lipoprotein cholesterol
LDLR	Low-density lipoprotein receptor
LIPC	Hepatic lipase
LPL	Lipoprotein lipase

LXRA	Liver X receptor alpha
MCFA	Medium chain fatty acid
MCP-1	Monocyte chemoattractant protein 1
MetS	Metabolic syndrome
MUFA	Monounsaturated fatty acid
NFKB1	NF-κB subunit 1
NPC1L1	Niemann-Pick C1-Like 1
NPR3	Natriuretic peptide receptor C
PCSK9	Protein convertase subtilisin kexin-9
PPARA	Peroxisome proliferator-activated receptor alpha
PPARD	PPAR delta
PPARG	PPAR gamma
PS	Plant sterol
PUFA	Polyunsaturated fatty acid
RBC	Red blood cells
RCFFN	Richardson Centre for Functional Foods and Nutraceuticals
SFA	Saturated fatty acid
SMOW	Standard mean ocean water
SNP	Single nucleotide polymorphism
SREBF2	Sterol regulatory element-binding factor 2
TC	Total cholesterol
TG	Triglyceride
TFA	*trans* fatty acid

TNFA	Tumor necrosis factor alpha
TRAF	TNF receptor-associated factor 3
UCP	Uncoupling protein

CHAPTER I

GENERAL INTRODUCTION

1.1 INTRODUCTION

The interplay between dietary behaviours and health outcomes is beyond debate, and has potential economic effects, at both the individual and population levels (1). Cardiovascular diseases (CVD), including coronary heart disease (CHD) and stroke, continue to lead the causes of morbidity and mortality worldwide, accounting for over 30% of all deaths in 2008 (2). The global economic burden associated with the management of CVD reached US $863 billion in 2010 and is projected to exceed $1 trillion by 2030 (2). Similarly, in Canada, 29% of all deaths in 2008 were secondary to CVD, which was estimated to cost upwards of CAD $21 billion in annual healthcare and related costs (3). Disturbances in lipid metabolism, also known as dyslipidemia, whether manifest as hypercholesterolemia, hypertriglyceridemia, high low-density lipoprotein cholesterol (LDL-C) concentrations, or low high-density lipoprotein cholesterol (HDL-C) concentrations, or a combination of these, exist as established risk factors for CVD (4). Adherence to dietary recommendations, typically advocating (within calorie needs) intakes of fruit and vegetables, whole grain products, low-fat dairy, legumes, lean protein foods such as seafood, and mono- and polyunsaturated fats rather than refined grains, red and proceed meat, and saturated and *trans* fats, represents one of the first lifestyle strategies targeting optimal lipid profile and cardiovascular health (5).

Despite this well-defined association, wide variability has been demonstrated across individuals in responsiveness of circulating cholesterol to dietary intakes (6-10), even with adjustment for confounders (11). Evidently, some individuals are hyper-responders to certain nutrients or dietary habits whereas others are non- or hypo-responders. This inter-individual variability possesses a genetic basis (12). Independent of diet, genetic factors account for about 40-60% of inter-individual variation in CVD risk, both directly and through influences on biomarkers, such as the lipid profile (13). Genome-wide association studies (GWAS) have identified many genetic loci involved in lipid metabolism, including circulating concentrations of total cholesterol (TC), LDL-C, HDL-C, and triglyceride (TG) (14-16). The role of these genetic determinants in the shaping of cholesterol concentrations and future prediction of CVD risk through interactions with nutrients has been the focus of scientific interest, especially following the emergence of Nutritional Genomics in the past decade (17). Public bioinformatics databases currently document millions of single nucleotide polymorphisms (SNPs), tens of which have been shown to have differing influences on health traits via interactions with lifestyle factors, such as diet. To date, the classical approach of such "gene-diet or gene-nutrient interactions" has been to explore single nutrients rather than whole foods or dietary behaviours in their entirety. The latter are, however, logically better in capturing more accurate views into eating habits of populations and can hence aid in better tailoring of personalized dietary advice, eventually leading to potential health and economic benefits for societies. One such whole food is dairy, meaning dairy products as a category.

Dairy is a nutrient-rich food group that is a significant source of an array of macro- and micronutrients, and other bioactive compounds. For this reason, dairy consumption is

recommended at the level of 2-4 servings/day, as part of an overall healthy eating pattern and within personal calorie needs, by most food guides worldwide in order to achieve optimal nutritional status (18-21). Accumulating evidence from epidemiological and dietary intervention studies further suggests that the consumption of milk and other dairy products may have beneficial effects on risk factors that contribute to CVD, including hypercholesterolemia, although data have been fairly conflicting (22-38). Many reviews and meta-analyses have underlined such data (39-54), leaving calls for a deeper look into the evidence for a better understanding of the contributing factors. Thus far, the rather discrepancy in research findings concerning the impact of dairy product intake on biomarkers of cardiovascular health has been largely thought to stem from factors related to study design and methodology, such as treatment duration, sample size and inclusion criteria, habitual dietary intake prior to intervention, compliance in terms of dietary protocols and physical activity, as well as number of servings and nature of the specific dairy products consumed. A shortcoming in most of the existed studies is the lack of assessment of biological markers of dairy intakes, such as certain odd-chained fatty acids (FA) specific to dairy fat consumption (55), as a tool to monitor compliance. Measuring such biomarkers of intake, rather than using food diaries, can contribute to better knowledge of an association between dairy consumption and health, especially in free-living trials.

Most, but not all, studies in the current literature show no or inverse association between the intake of milk fat, and in some cases full-fat dairy products, and CVD risk (51). As for the impact on circulating cholesterol concentrations, of all dairy products, butter has been the least controversial, when substituted for carbohydrates or unsaturated fatty

acids, given the consistent associations between butter intake and increased LDL-C and/or HDL-C concentrations (24, 26, 32). The literature on milk and fermented dairy products, such as yogurt and cheese, is less conclusive but mostly indicative of protective effects on both cholesterol concentrations (39, 40, 42, 49) and cardiovascular health (41, 47, 51, 54, 56-59), especially when compared with butter intake of equal total fat and saturated FA content. Such observations can be attributed to a net balance between positive and negative cholesterolemic effects of dietary bioactives in dairy; saturated fatty acids (SFA) and calcium are examples of nutrients that may differently modify the effects of certain dairy foods on cholesterol. Independent of the dietary factors, however, and in light of previous research in the nutrigenetics arena with foods that resemble dairy, such as those high in dietary cholesterol (60, 61) and SFAs (62, 63), a causal gene-nutrient (or gene-diet) interaction is likely a powerful contributor to the observed differing impacts of dairy product intake on cholesterol metabolism.

To date, less than a handful of studies have attempted to reveal potential interactions between the dairy food group and genetic variants in relation to certain biomarkers of health (64-66). However, to our knowledge, no research has looked into common variants within genes that encode key metabolites in pathways of cholesterol metabolism as they relate to cholesterol homeostasis, including circulating concentrations and absorption or synthesis rates in response to dairy. Therefore, investigating the inter-individual variability in relation to the responsiveness of cholesterol metabolism, as a driving factor of circulating concentrations, to dairy consumption is highly warranted. Such approach is anticipated to provide novel knowledge in the gradual transitioning to a personalized nutritional strategy of dietary interventions, dictated by an individual's genetic make-up,

which would emphasize the importance of dairy products from individual to individual within a population.

On the other hand, and apart from the association with cholesterol metabolism, cardiovascular events can occur in people with normal cholesterol concentrations, and even among those with no major risk factors (67). The presence of an underlying pro-inflammatory state, usually characterized by increased circulating concentrations of C-reactive protein (CRP) and inflammatory cytokines (e.g. IL-6 and TNF-α), as well as by decreased concentrations of anti-inflammatory mediators such as adiponectin, represents an independent risk factor for CVD (68). A better assessment of the CVD risk can thus be achieved by measuring the inflammatory state in addition to the lipid profile. Findings from a majority of cross-sectional studies investigating the effects of low-fat dairy on inflammatory markers, as part of a healthy diet, suggest an inverse association (69-72). However, randomized controlled trials specifically designed to assess such inflammation-related outcomes in response to a recommended dairy consumption are largely lacking.

Overall, additional human intervention studies are needed to further the understanding of effects of the recommended level of a blended dairy consumption on classical and emerging biomarkers of cardiovascular health, and to elucidate the potential mechanisms of action from both the metabolic and genetic perspectives.

1.2 RATIONALE

Dairy foods represent a complex array of nutrients that, individually and in certain clusters, show differing effects on established and emerging biomarkers of cardiovascular

health, including concentrations of circulating cholesterol and inflammatory mediators. Studies examining the association between dairy intake and these biomarkers have returned a contradictory picture of evidence. Daily consumption of dairy products is generally recommended, yet a few randomized controlled studies have looked at impacts of the "recommended" intake level of conventional dairy products, as a whole versus no dairy, on circulating concentrations of cholesterol and inflammatory mediators. Indeed, no such studies have occurred in Canada where 2-3 servings/day of dairy are recommended. Moreover, dairy foods are rich sources of a large array of FAs that can individually offer varying effects on cholesterol. However, no particular crossover study has examined the impact of the recommended dairy intake on circulating FA profile, or related it to cholesterol responses. The potential act of certain FAs in dairy fat, such as medium chain FAs (C6:0-C12:0), C15:0, and C17:0, as potential biomarkers of intake has not been established in dietary interventions of short durations. From a mechanistic view, whether or not conventional dairy relative to non-dairy products alter cholesterol absorption, biosynthesis, or clearance rates have also not been investigated. Furthermore, to date, no gene-nutrient interaction studies have specifically investigated the influence of inter-individual variability in cholesterol-related genes on the response of cholesterol homeostasis to the intake of dairy foods. As such, an obvious need exists to understand, assess, and identify the possible metabolic and genetic factors that may explain, at least in part, the reported inconsistency in the degree of responsiveness of cholesterol metabolism, and other metabolic risk parameters, to dairy consumption.

Using a multicentre, free-living, randomized, crossover, controlled human intervention design, the primary focus of the present research is to delineate the impact of common

genetic variants in candidate genes along known cholesterol metabolic pathways on responses of cholesterol metabolism, including circulating cholesterol concentrations and rates of cholesterol absorption and biosynthesis, to the recommended level of dairy consumption in Canada. A secondary focus is to explore the impact of dairy intake on biomarkers of inflammation and elucidate effects on the expression of inflammatory genes. Healthy normocholesterolemic men and women, with and without low-grade systemic inflammation, defined as increased circulating concentrations of CRP, incorporated 3 servings/day of low-fat and regular milk, yogurt, and cheese, or dairy-free energy-equivalent control products from the local market into their everyday diet for 4 weeks each, as part of a prudent background dietary protocol. The research took place concurrently at the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN) in Winnipeg, Manitoba, and the Institute of Nutraceuticals and Functional Foods (INAF) in Laval, Quebec. Whereas lipidomics represented the major research theme at the RCFFN, inflammation was investigated with special focus at the INAF. The output of this research is fundamental in advancing the knowledge of the genetic basis by which cardiovascular health may be modulated, particularly through the cholesterol responsiveness to consumption of conventional low-fat and regular dairy products.

1.3 OBJECTIVES

The overarching purpose of this research programme is to extend knowledge on the recommended level of dairy consumption in Canada in relation to circulating cholesterol and inflammatory biomarker concentrations, with particular focus on identifying a

genetic basis for any heterogeneity in responsiveness of cholesterol metabolism to dairy intake. Specific objectives include:

1. Investigate the impact of a recommended 3 versus 0 servings/day intake of conventional and commonly-consumed dairy products on risk factors for CVD, including circulating lipid and inflammatory biomarkers.
2. Evaluate the influence of the recommended dairy intake on circulating FA profile, including the capacity of certain FAs specific to dairy fat to serve as potential short-term biomarkers of intake.
3. Associate common variants within candidate genes involved in cholesterol metabolism with the degree of circulating cholesterol and TG responsiveness to the recommended dairy intake.
4. Examine the impact of the recommended dairy intake on cholesterol homeostasis, as determined by circulating surrogate non-cholesterol sterol markers and isotope tracer techniques.
5. Associate common variants within candidate genes involved in cholesterol metabolism with changes in cholesterol enrichment and fractional synthesis rate, as characterized by stable isotope tracers, in response to the recommended dairy intake.

1.4 HYPOTHESES

The hypotheses to be tested include:

1. Consumption of dairy products of this research will not adversely modify circulating concentrations of lipid or biomarkers of inflammation in a short-term.
2. Consumption of the dairy products will modify circulating FA profile in a manner that enables detection of FA biomarkers specific to dairy fat.
3. Heterogeneity in candidate genes with functional links to cholesterol will associate with the degree of circulating cholesterol responsiveness following the dairy intake.
4. Consumption of the dairy products, relative to control, will increase circulating surrogate markers of cholesterol absorption but not synthesis rate.
5. Heterogeneity in candidate genes with functional links to cholesterol will associate with the degree of cholesterol enrichment and fractional synthesis rate following the dairy intake.

1.5 OUTLINE OF THIS THESIS

This thesis was written using the manuscript style and is composed of five manuscripts that follow after the General Introduction (Chapter I) and a Mini-Literature Review (Chapter II). Chapter II summarizes data of the recent literature surrounding the effects of dairy consumption on circulating cholesterol, FA profile, and inflammatory markers from human studies, as well as effects of dairy on CVD risk as a hard endpoint measure. The first manuscript (Chapter III) presents, in a systematic approach, findings derived from

observational and dietary intervention studies associating genetic variants in the genes along known cholesterol metabolic pathways with fasting cholesterol concentrations in interaction with dietary intakes. Thereafter, the research manuscripts addressing each specific objective in this thesis are presented, providing insights into the recommended level of dairy intake in Canada in relation to circulating cholesterol and FA concentrations (Chapter IV), genetic basis for heterogeneity in responsiveness of circulating cholesterol (Chapter V) and cholesterol metabolism (Chapter VI), and, finally, dairy intake in relation to systemic inflammation, including expression of inflammatory genes in whole blood cells (Chapter VII). Manuscripts 1, 2, and 5 have been published, manuscript 3 has been accepted provisionally and is currently under a final peer-review process, and manuscript 4 is in submission. Bridges in between manuscripts link the chapters for a consistent flow of the thesis. Chapter VIII (General Discussion) provides an overall summary of the thesis with concluding remarks and implications, novelty of the work, and limitations and future directions.

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BRIDGE TO CHAPTER II

The following chapter provides an overview of the current state of knowledge surrounding the association of dairy intake, in epidemiological and clinical studies, to circulating lipid and inflammatory mediators as examples of established and emerging biomarkers of cardiovascular health, respectively. The inconsistency in research observations attributed to several lifestyle and research-oriented factors is defined, and the potential contribution of genetic factors to such observations is highlighted. In general, this chapter is an introduction to the topic of dairy intake as it relates to the cardiovascular disease risk factors of interest in this thesis.

CHAPTER II

MINI-LITERATURE REVIEW

DAIRY PRODUCT CONSUMPTION AND CARDIOVASCULAR HEALTH

2.1 INTRODUCTION

Several studies, including meta-analyses, have examined the impact of milk and other dairy product consumption, and functional nutrients of dairy such as calcium and fatty acids (FA), on traditional and emerging biomarkers of chronic disorders. Elevated circulating cholesterol concentrations and systemic inflammation are well-defined examples of these biomarkers in the progression of metabolic syndrome (MetS) and cardiovascular disease (CVD). Data of the association between dairy consumption and these biomarkers have been largely inconclusive, however. The purpose of the following sections is to provide a brief summary of the current state of knowledge surrounding impacts of dairy product intake on cholesterol metabolism, FA profile, and inflammatory state in epidemiological and human dietary studies. The potential role of the genomic architecture particularly in modulating the cholesterol responsiveness to dairy is also discussed.

2.2 CIRCULATING CHOLESTEROL CONCENTRATIONS AND CARDIOVASCULAR HEALTH

Coronary heart disease (CHD) and stroke, the two constituents of CVD, continue to lead the causes of morbidity and mortality worldwide (1) accompanied by significant

economic burdens on healthcare and productivity (2, 3). Deregulated lipid metabolism, when manifests as dyslipidemia and characterized by high concentrations of total cholesterol (hypercholesterolemia), high low-density lipoprotein cholesterol (LDL-C), low high-density lipoprotein cholesterol (HDL-C), high triglycerides (hypertriglyceridemia) in blood, or a combination of these features, is an established risk factor for CVD (4). In epidemiological and meta-analysis studies, a 10% (or 0.6 mmol/L) reduction in serum TC concentrations has been associated with a 20-50% lower risk of CHD, and a 25-30% reduction in CHD-related mortality over long terms (5). Similarly, a 10% reduction in LDL-C concentrations has been associated with a 20% reduction in cardiovascular events (5). Also, a 2-3% decrease in risk for future CHD has been demonstrated with as low as 0.03 mmol/L increase in HDL-C concentrations (6).

Lifestyle has been identified as a major player in the development, progression, and management of lipid profile and cardiovascular health, where consuming an overall healthy diet is a first strategy for targeting optimal states (7). Such diet partly centers around limiting intake of saturated fat (<7% of energy), *trans* fat (<1% of energy), and cholesterol (<300 mg/day) by choosing lean meats and vegetable alternatives, selecting fat-free or low-fat dairy products, and minimizing intake of partially hydrogenated fats, within the range of 25% to 35% of energy from total fat intake (8).

2.3 DAIRY PRODUCTS: RECOMMENDED AND CURRENT INTAKES

Dairy foods, for which bovine milk is by large the base material, have been recognized as an integral component of the human diet for millennia. Because it plays an important part in health by providing essential nutrients for growth and body maintenance throughout

lifespan, dairy consumption is recommended usually at the level of 2-4 servings/day by food guides in most Western countries (9-12). In Canada, 'Milk and alternatives' is one of four basic food groups of Canada's Food Guide, which recommends that adults drink 2-3 servings/day of low fat milk [skim, 1% milk fat (M. F.), or 2% M. F.] or consume lower fat milk alternatives, such as yogurt, cheese, and fortified soy beverages each day (9). It is stated that this directive would be "an effective way to consume protein, calcium, magnesium, riboflavin, vitamin A, vitamin B12, vitamin D and zinc while minimizing the amount of saturated fat and calories" (9).

The range of milk and dairy products varies among countries depending on dietary habits, processing technologies, market demand, and social and cultural factors. The per capita consumption of these products is generally higher in developed versus developing countries (13). Also, the demand has been for liquid milk in urban centres and for fermented products in rural areas, although processed products are now becoming increasingly common in many regions (13). Similar to many parts of the world (14-17), in Canada, dairy products are under-consumed by all age groups, with an average daily intake of 1.24 to 1.95 servings in adults (18).

Evidently, the nutritional composition of dairy products varies depending on the food matrix and factors such as the feed composition, breed and age of the animal (e.g. cow), stage of lactation, season, and health status of the udder (19). On the whole, dairy foods are known as a major source of saturated fatty acids (SFAs) that generally account for ~75% of total dairy fat, which, in turn, have traditionally been associated with higher cholesterol concentrations (20) and, hence, increased risk for CVD (21). This has recently

been a subject of much debate, however (22, 23). Dairy is also a source of nutrients including vitamin D, potassium, phosphorus, and calcium that might counterbalance the potentially unfavorable physiologic effects of SFAs (24, 25). As such, data associating dairy intakes to circulating cholesterol concentrations and other biomarkers of cardiovascular health paint a somewhat conflicting picture of evidence (26-41), leaving questions in regards to contributing effects of lifestyle, metabolic, and, possibly, genetic factors based on the available evidence.

2.4 EFFECT OF DAIRY PRODUCT INTAKE ON CIRCULATING CHOLESTEROL AS A BIOMARKER OF CARDIOVASCULAR HEALTH

2.4.1 Epidemiological studies

The association of dairy intake with circulating cholesterol concentrations has long been addressed in epidemiological and dietary intervention studies, and summarized in review articles (42-45). For the past few decades most, but not all, of those studies have suggested that the consumption of milk and fermented dairy products may have a cholesterol-improving potential (26). Relevant epidemiological studies, by design, examine dairy as a group of foods in the habitual diets of populations. Also, such studies often present the lipidemic/cholesterolemic effect of dairy intake as part of the MetS features, including central obesity, insulin resistance, and hypertension. For instance, the Coronary Artery Risk Development in Young Adults (CARDIA) cohort, involving 3,157 adults, showed inverse associations between dairy intake and MetS (42). Over 10 years, the adjusted odds ratio (OR) of developing MetS was 72% lower (OR = 0.28, 95% CI 0.14-0.58) among overweight individuals in the highest (≥35 times/week) versus lowest

(<10 times/week) category of dairy intake. There, the OR for dyslipidemia as represented by odds of higher circulating TG concentrations was 21% lower (OR = 0.79) for each daily increment of total dairy intake (42). Similarly, in an Iranian cross-sectional study involving 827 adults, participants in the highest quartile of dairy intake (≥3.1 servings/day) showed lower adjusted odds for MetS (OR = 0.82, 95% CI 0.63-0.99) and serum TG concentrations (OR = 0.90, 95% CI 0.74-1.10) (44). Also, among a representative sample of 2,375 males in the Caerphilly prospective study, the adjusted OR for MetS in individuals who habitually drank more milk per day was 0.38 (95% CI 0.18-0.78) and that for dairy intakes was 0.44 (95% CI 0.21-0.91) relative to those who drank little or no milk at all (45). Similar data have been highlighted in a 2011 systematic review of 10 cross-sectional and 3 prospective cohort studies, which suggested an overall potential benefit of dairy, including milk, yogurt, and cheese intake, on the risk of having MetS (37).

2.4.2 Dietary intervention studies

Unlike epidemiological studies, which typically examine dairy foods as part of habitual dietary intakes and associate them with an overall risk for chronic conditions, such as the MetS and CVD, dietary interventions 'by design' usually test and compare individual dairy products as they relate to certain biomarkers of health, such as the lipid profile. For instance, a crossover study involving 8 males who consumed 660 mL/day of skim or whole milk, on a healthy-style isocaloric background diet for 6 weeks, showed greater decreases in plasma concentrations of TC (4.5 *vs.* 4.8 mmol/L) and LDL-C (2.6 *vs.* 3.0 mmol/L) with the intake of skim versus whole milk (46). Another study (47) compared

standard skim milk with that from cows immunized against certain human intestinal bacteria in 11 hypercholesterolemic males and found an 8% (~0.5 ± 0.6 mmol/L) reduction in serum TC concentrations when the immune milk was consumed in ≤0.3-0.5 L/day for 8 weeks. This observation was reproduced a few years later in a double-blind crossover trial with 30 hypercholesterolemic individuals who consumed 90 g/day of immune milk (in a powder form) or ordinary skim milk for 10 weeks (48). There, again, decreases in plasma TC and LDL-C concentrations by 5.2% and 7.4% were respectively reported with the consumption of the immune compared with control milk. On contrary, Thompson et al. (49) assigned 6 groups of 10 to 13 healthy participants to receive either one of four control milks, with varying fat contents, versus yogurt and buttermilk containing different bacterial strains. After 3 weeks of intake, the fermented dairy products did not influence serum lipid concentrations differently than the control milks. In another parallel design involving 78 adults, de Roos et al. (50) also failed to detect differences in serum lipid concentrations after intake of 500 mL/day of control or *Lactobacillus acidophilus*-enriched yogurts for 6 weeks.

Of all dairy foods in more recent dietary intervention studies, the influence of butter on circulating cholesterol concentrations has been the least controversial (29), whereas data of milk, yogurt, and cheese intakes were less conclusive. For example, in a 4-week crossover design involving 19 participants (13 of whom were hypercholesterolemic), daily intake of 40 g dairy fat (17% en SFA) as butter, but not as matured cheddar cheese, resulted in higher plasma TC concentrations (6.1 ± 0.7 mmol/L for butter and 5.8 ± 0.6 mmol/L for cheese) versus a diet containing less saturated fat (12.5% en) (5.6 ± 0.8 mmol/L) (51). In the same study, butter intake increased plasma LDL-C concentrations

(4.4 ± 0.3 mmol/L) compared to cheese (3.9 ± 0.3 mmol/L) only among the hypercholesterolemic participants, whereas no differences were observed with HDL-C or TG concentrations. In a strictly controlled crossover design of 3 weeks, Tholstrup et al. (52) previously provided 20% of total energy intake per day as whole milk, butter, or hard cheese, with similar amounts of fat, to 14 healthy young males. Plasma LDL-C but not other lipid concentrations were higher after butter relative to the cheese intake, as well as after milk and butter intakes, but not cheese, compared to the baseline diet (52). The effects of yogurt on circulating cholesterol concentrations in many studies have also been documented (29). Intakes of 300 g/day of probiotic and conventional yogurts were investigated in a 6-week double-blind controlled trial involving 60 diabetic Iranian individuals (53). Whereas no changes in serum HDL-C or TG concentrations were observed, there, intakes of the probiotic yogurt led to 5.5% and 7.5% reductions in TC and LDL-C concentrations, respectively, as well as to reductions in TC/HDL-C and LDL-C/HDL-C ratios in comparison with the conventional yogurt (53). On the other hand, in a controlled trial of calcium supplements involving 1,080 elderly Australian females, those who consumed ≥200 g/day of yogurt, but not other dairy products, at baseline showed higher (1.5 ± 0.4 *vs.* 1.4 ± 0.4 mmol/L) blood HDL-C concentrations in comparison to consumers of <200 g/day of yogurt (54). The findings were consistent with those previously revealed in a crossover trial involving 29 healthy females, where 300 g/day of probiotic yogurt increased serum HDL-C but not TC or LDL-C concentrations by 0.3 mmol/L over 21 weeks of intake (55). Most of other interventions that examined dairy as a group of products did not observe effects on circulating cholesterol concentrations (56-59). For instance, a 21-week intake of 4 daily servings of dairy products in the forms of

low-fat milk, cheese, and yogurt that provided a median of 1187 mg/day of dietary calcium, versus a control diet (median 691 mg/day of calcium), resulted in 0.1 and 0.2 mmol/L non-significant increases in TC and LDL-C concentrations, respectively (57). Similarly, another crossover trial undertaken in 61 overweight or obese adults who were assigned to a high dairy diet (4 servings/day of reduced fat dairy) or a low dairy control diet (≤1 serving/day) for 6 months each showed no significant differences in cholesterol concentrations or any other cardiometabolic parameters (59).

Indeed, the above differing observations may be attributed to the nutritional compositions of dairy products employed in research, or in the wide spectrum of experimental designs and populations of study. Although yet to be fully disclosed, the potential role of genetic factors in this context cannot be completely ruled out, however.

2.5 THE GENETIC FACTOR IN THE IMPACT OF DAIRY INTAKE ON CHOLESTEROL METABOLISM

With the official completion of the Human Genome Project in 2003, a substantial number of DNA sequence polymorphisms were revealed. The most widespread type of these is the single nucleotide polymorphism (SNP), defined as a single base pair mutation at a specific locus on the DNA sequence of a given gene. Public bioinformatics databases currently document millions of SNPs that show differing influences on health traits. As far as the CVD is concerned, genetic factors have been estimated to account for 40-60% of inter-individual variation in risk, both directly and through influences on biomarkers such as the lipid profile (60). Genome-wide association studies (GWASs) have identified over 150 distinct genetic loci that are involved in shaping circulating cholesterol

concentrations (61-63). The impacts of SNPs within genes encoding metabolites, such as transporters, along the cholesterol metabolic pathways in association with nutrient intakes have also been reported (64, 65). To date the classical approach of these gene-diet interactions has been to explore single nutrients rather than whole foods or dietary behaviours in general. The latter are, however, typically better in capturing more accurate views into eating habits of populations and can hence aid in better tailoring of personalized dietary advice, eventually leading to potential health and economic benefits.

Recently, SNPs within certain genes of interest have been investigated for potential interactions with dairy diets in three studies (66-68); at present, to our knowledge, these are the only gene-diet interaction studies that involve dairy intakes. In the first study, carriers of the lactase (*LCT)* gene SNP rs4988236-C/C genotype showed lower body weight, body mass index (BMI), and waist circumference, but no change in lipid concentrations, following intakes of dairy (66). In the second, apolipoprotein A2 (*APOA2*) SNP rs5082-C allele was associated with greater BMI on a higher versus lower intake of high-fat dairy foods in 2 U.S. populations (67). And, in the third study, carriers of the peroxisome proliferator-activated receptor alpha (*PPARA*) SNP rs135549-T/T genotype, but not the C allele, had lower TC/HDL-C and LDL-C/HDL-C ratios after a 12 month intake of skim milk (68). Studies assessing impacts of common SNPs within cholesterol-related genes on responsiveness of cholesterol metabolism to dairy consumption are lacking, however. Still, in light of data from research with other dietary regimens that resemble dairy foods in some aspects, such as those characterized by higher dietary cholesterol and SFAs, a casual genotype-diet interaction can be expected. For instance, in a cross-sectional study involving 35 French Canadian males, higher intake

(>290 mg/day) of dietary cholesterol was associated with 9.8% and 10.5% higher plasma TC and LDL-C concentrations, respectively, only in carriers of the *LXRA* SNPs rs12221497-A, rs61896015-A, and rs3758674-C alleles, in comparison to carriers of other genotypes (69). LXR (liver X receptor) is a transcription factor involved in cholesterol, FA, and glucose metabolism. Another cross-sectional trial of 23,011 individuals associated higher (>14.3% en) intakes of SFAs with increased serum TC and LDL-C concentrations (6.2 and 4.0 mmol/L, respectively) in carriers of the *HMGCR* SNP rs17238540-T/T but not other genotypes (70). HMGCR (3-hydroxy-3-methylglutaryl-coenzyme A reductase), the target of the cholesterol-lowering drugs statins, is a rate-limiting enzyme involved in endogenous synthesis of cholesterol. A third cross-sectional study involving 1,227 Spanish children previously indicated that with lower SFA (14.3-34.1 g/day) intakes, only male carriers of the *ABCG5* SNP rs6720173 minor allele G had higher plasma TC (4.9 *vs.* 4.5 mmol/L, respectively) and LDL-C (3.1 *vs.* 2.6 mmol/L, respectively) concentrations compared to the C/C homozygotes (71). In the same study, *ABCG8* SNP rs6544718-C>T heterozygotes showed higher TC concentrations (4.8 and 5.1 mmol/L for males and females, respectively) compared to the C/C homozygotes (4.6 mmol/L for males and females) only when cholesterol intake was less than 300 mg/day. These findings suggest that effects of dietary cholesterol and SFA intakes on cholesterol concentrations may be modulated by polymorphisms within ATP-binding cassette sub-family G5 (*ABCG5*) and -8 (*ABCG8*) genes, both of which encode transporters responsible for phytosterol and cholesterol excretion from enterocytes into intestinal lumen, and from hepatocytes into the bile. Polymorphisms within genes encoding other metabolites that are involved in cholesterol absorption, synthesis, or transport have also

been repeatedly linked to cholesterol responses in associations with other dietary modifications in gene-diet interaction studies (72-79). The majority, if not all, of these studies relied on food diaries rather than biological markers as a means of assessing intakes and verifying compliance to dietary protocols.

2.6 FATTY ACIDS AS BIOMARKERS OF MILK AND DAIRY FAT INTAKE

Dietary biomarkers are desirable over self-reported food assessment methods for their ability to better reflect food intakes and nutritional status. Circulating FAs represent some of the best available biomarkers of dietary fat intakes, offering an objective assessment versus food recalls (80, 81). The lipid fraction of milk consists predominantly of TG (>95%) and contains more than 400 individual FAs, most of which are present in amounts of <1% of total lipids (82). Epidemiological studies have examined certain circulating FAs as biological markers for dairy intake, and demonstrated an association in most but not all cases (83-90). In particular, two odd-chain SFAs, pentadecanoic acid (C15:0) and heptadecanoic acid (C17:0), have been of major interest. These FAs cannot be synthesized in the human body and are specific to dairy fat and ruminant meat owing to their synthesis by bacterial flora in the rumen of bovine species (91). In one study, healthy men aged 21-55 years provided serum (n = 110) and adipose tissue samples (n = 107), and completed both 14 days weighed records (WR) and a 180-item food frequency questionnaire (FFQ). The strongest correlation coefficients (r) were observed between total intake of dairy fat estimated from WR and relative content of C15:0 in adipose tissue (0.52, 95% CI 0.37-0.65) and total serum (0.43, 95% CI 0.26-0.57). A consistent inverse association was observed between the intake of milk fat and relative serum

content of C17:0 ($r = -0.26$), however (86). Another direct association ($P \leq 0.005$) was found between dairy product consumption and serum C15:0 ($r = 0.13$) and C17:0 ($r = 0.10$) concentrations in a cross-sectional study of 1,114 healthy women who completed diet history questionnaires and supplied blood samples in the French component of the EPIC cohort (89). Also, data from the population-based Vasterbotten Intervention Project in Sweden, including 96 men and 99 women, showed significant correlations between FFQ-estimated intakes and erythrocyte membrane contents of the milk FAs C14:0, C15:0, and C17:0 ($r = 0.23$-0.34, $P \leq 0.05$) (88). Such associations between dairy products consumption and biomarkers of intake in controlled clinical trials are largely missing.

2.7 BIOMARKERS OF DAIRY FAT AND ASSOCIATION WITH CARDIOVASCULAR DISEASE

A few case-control studies have been conducted to associate certain FAs, as potential biomarkers of dairy fat intake, in circulation or adipose tissue with positive (35, 87) and negative (92) effects on CVD risk. Biong et al. (87) studied 99 patients who survived myocardial infarction (MI) and 98 controls, and found contents of FAs C14:0 (3.72 *vs.* 3.47 mol/100 mol, $P = 0.04$), C15:0 (0.41 *vs.* 0.37 mol/100 mol, $P = 0.01$), and C17:0 (0.27 *vs.* 0.26 mol/100 mol, $P = 0.05$) to be higher in adipose tissue of controls than in patients. Further, age and sex adjusted odds ratios (OR) for MI were significantly reduced with increasing quartiles of these FAs in adipose tissue, but except for C15:0 (OR = 0.36, 95% CI 0.13-0.99), the trend was no longer significant after adjustment for other confounders. Warensjo et al. (90) more recently conducted another prospective case-

control study with 444 MI cases and 556 controls. There, proportions of C15:0, C17:0, and C15:0 + C17:0 in plasma phospholipids were all significantly higher in woman (but not men) controls than in cases, with a 26% lower risk of becoming an MI case in women and 9% lower in men. Further, in agreement with the biomarker data, quartiles of reported intake of cheese (women and men) and fermented milk products (men) were inversely related to a first MI ($P < 0.05$). This study, therefore, concluded that milk fat biomarkers are inversely associated with risk of developing a first MI, especially in women. On the other hand, among 166 incident cases of ischemic heart disease (IHD) and 327 controls from the Nurses' Health Study, multivariate-adjusted relative risks (95% CI) from the lowest to highest tertile of C15:0 concentrations in plasma were 1.0 (reference), 2.18 (1.20-3.98), and 2.36 (1.16-4.78) ($P = 0.03$) among IHD cases (92). Accordingly, this study was the only to find increased risk of CVD events associated with circulating FA C15:0 concentrations. Finally, a 2014 meta-analysis of data from 4 cohort studies involving 5,490 participants showed an association only between higher circulating C17:0 concentrations and 23% lower CHD risk (93). As such, findings of these studies should be taken with caution awaiting additional evidence and thorough assessment of the FA biomarkers of dairy fat intake in controlled dietary intervention studies.

2.8 EFFECTS OF DAIRY INTAKE ON THE INFLAMMATORY STATE

Low-grade systemic inflammation is a nontraditional risk factor in the development and progression of several chronic disorders, including atherosclerosis (94), MetS (95), and CVD (96). Elevated circulating concentrations of C-reactive protein (CRP) and the pro-

inflammatory cytokines IL-6 and TNF-α have in particular been associated with cardiovascular events (97-100). Increasing interest in the health implications of dairy in recent years has stimulated research into these biomarkers of inflammation and endothelial cell function, with observational and experimental research findings suggesting inverse associations (101-104). For instance, among 1,514 men and 1,528 women from the ATTICA study in Greece, Panagiotakos et al. (103) have identified an inverse relationship between dairy consumption and concentrations of various inflammatory biomarkers. Specifically, CRP, IL-6, and TNF-α concentrations in individuals consuming more than 14 servings of dairy products per week (i.e. >2 servings/day) were 29%, 9%, and 20% lower, respectively, than in those consuming less than 8 servings (≤1 serving/day), independent of confounders such as age, sex, smoking, physical activity, body mass, and other dietary habits. Also, in a 2013 systematic review of eight randomized controlled dietary interventions, Labonte et al. (105) assessed consumption of dairy products (i.e. milk, yogurt, and/or cheese) in relation to biomarkers of inflammation in adults, and underscored no adverse effects. There, one study had identified improved pro- and anti-inflammatory biomarker concentrations following high- versus low-dairy diets, three studies showed improvement in CRP, IL-6, or TNF-α after dairy, and four studies showed no effect. In light of some limitations in the available evidence as outlined by the authors of the systematic review (105), however, further controlled dietary interventions are warranted to substantiate the effects of dairy intake on inflammation-related outcomes and elucidate the underlying mechanisms of action.

2.9 CONCLUSION

In light of the available evidence, dairy consumption is associated with rather varying impacts on biomarkers of cardiovascular health, particularly circulating cholesterol and inflammatory mediator concentrations, apparently due to differences in the nature of products employed in research or of study designs. The genomic architecture is likely a contributing player that is presently under-documented, however. As such, controlled dietary intervention studies specifically designed to address the genetic basis that may contribute to the existing picture of evidence in the association of dairy product intake to cholesterol and inflammation metabolism, while verifying compliance through established biomarkers of intake, are warranted.

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BRIDGE TO CHAPTER III

Chapter III comprises a systematically-driven narrative review of scientific evidence established in the post-genomic era, where several genetic variants within key cholesterol-related genes are identified as modulators of circulating cholesterol concentrations under diverse dietary scenarios. These findings of gene-diet interactions are, in essence, the basis that leads to the research initiative and project presented in Chapters V and VI. The following manuscript has been published in the Nutrition Reviews 2015;73(8):523-43.

CHAPTER III

MANUSCRIPT 1: LITERATURE REVIEW

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NUTRIGENETICS OF CHOLESTEROL METABOLISM: OBSERVATIONAL AND DIETARY INTERVENTION STUDIES IN THE POSTGENOMIC ERA

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3.1 ABSTRACT

Cholesterol metabolism is a well-defined responder to dietary intakes and a classic biomarker of cardiovascular health. For this reason, circulating cholesterol levels have become key in shaping nutritional recommendations by health authorities worldwide for better management of cardiovascular disease, a leading cause of mortality and one of the most costly health problems globally. Data from observational and dietary intervention studies, however, highlight a marked between-individual variability in the response of cholesterol metabolism to similar dietary protocols, a phenomenon linked to genetic heterogeneity. This review summarizes the postgenomic evidence of polymorphisms within cholesterol-associated genes relative to fasting circulating cholesterol levels under diverse nutritional conditions. A number of cholesterol-related gene-diet interactions are confirmed, which may have clinical importance, supporting a deeper look into the rapidly emerging field of nutrigenetics for meaningful conclusions that may eventually lead to genetically targeted dietary recommendations in the era of personalized nutrition.

3.2 INTRODUCTION

The completion of the Human Genome Project in 2003 enabled the development of various techniques to characterize common genetic traits (1), leading to significant progress in genetic association studies in common and complex phenotypes, such as circulating cholesterol levels (2). Through various approaches including pedigree, epidemiological, candidate gene, and genome-wide association studies (GWASs) it has been established that genetic variations play a role in shaping the circulating levels of cholesterol, which are classic biomarkers for cardiovascular disease (CVD) (2-5). For example, as of April 2014, GWASs reported 155 distinct genetic variants associated with cholesterol metabolism (6,7). Most of these GWASs were, however, designed to identify variants associated to intrinsic circulating cholesterol levels without considering the possible interactions with particular diets or nutritional interventions.

The emerging field of nutrigenetics aims to determine how an individual's genotype controls the response of health biomarkers to dietary intakes, a phenomenon termed gene-diet interaction (8). The long-term vision is to develop genotype-specific personalized dietary recommendations for the prevention and treatment of chronic disorders. The challenge has, since, been to develop methods and approaches to effectively achieve these goals. Current research aims to determine gene-diet interactions through prospective studies and controlled dietary interventions.

This review summarizes the current knowledge on cholesterol-related genetic variations in association with responses of fasting circulating cholesterol levels to various dietary regimens in epidemiological and intervention studies. Evidence on the functional

mechanisms of these associations remains for the most part unknown and is, therefore, not presented. Future research directions essential to validate the present gene-diet interactions as a basis for personalized nutritional recommendations are discussed.

3.3 CURRENT STATE OF KNOWLEDGE

Database searches using PubMed were carried out in a systematic approach to identify cholesterol-oriented gene-diet interaction studies published in the English-language literature between January 01 2003 (the year of the Human Genome Project's official completion) and December 31 2013, with no restriction on the basis of study design, population, or quality throughout the preliminary search. With an initial search that included the keywords "cholesterol", "gene", "diet", and "interaction" combined, 173 articles were identified, many of which reported more than one gene and/or genotype x diet interactions. A follow-up search included the previous keywords combined in addition to the name of each of the 28 cholesterol-related gene of interest, presented in full names and abbreviations above. The resulting articles were then examined individually to exclude research reports that did not investigate fasting circulating cholesterol responses to gene-diet interactions, did not present clear associations, or did not provide research details (i.e. when data were not shown). Citation lists of the eligible articles were also screened to ensure the coverage of data from all related studies. Review-style, animal study, and non-English articles were excluded. A total of 69 distinct research articles were at the end eligible for inclusion in this review following abstract screening and study design evaluation. Finally, associations of within-person variability to cholesterol metabolism through factors other than diet, such as physical activity (9),

alcohol intake (10), smoking (11), pharmacological interventions (12), anthropometric features (13), or certain disease states (14,15), although do exist, reach beyond the scope of this review and are hence not covered.

The following paragraphs present data for gene-diet interactions reported in the cholesterol metabolic pathways in alphabetical order of the genes identified. Significant findings of the observational research and dietary intervention studies are summarized in **Tables 3.1** (16-41) and **3.2** (42-64), respectively.

3.3.1 ABCA1 polymorphisms modulate circulating HDL-C responses to low saturated fat and carbohydrate intakes

ATP-binding cassette sub-family A1 (ABCA1) is a membrane protein involved in the formation of high-density lipoprotein (HDL) particles and, as a cholesterol efflux pump, in the reverse cholesterol transport (65). Polymorphisms in the *ABCA1* gene have been shown to affect circulating HDL cholesterol (HDL-C) levels and, as a result, the incidence of CVD (66).

Following a prospective cohort design with instructions for a 12-week low saturated fat diet intake, including 8-week of 25 g soy protein and 15 g soluble fibre intake daily, hyperlipidemic Mexican individuals who were heterozygotes for the *ABCA1* single nucleotide polymorphism (SNP) rs9282541-C>T (published as R230C) genotype experienced higher (P = 0.05) serum HDL-C levels in comparison to the study's C/C (R230R) homozygotes (+14.6% *vs.* +4.6% change from baseline, respectively) (42). This

Table 3.1 Summary of observational studies with significant impact of gene-diet interactions on circulating cholesterol levels

Reference	Gene	Polymorphism(s)	Study design	Diet	Population (n)	Ethnicity	Cholesterol response[1]
Romero-Hidalgo et al. (2012) (16)	*ABCA1*	rs9282541	Cross-sectional	CHO	3,591 M and F	Mexican	↑ HDL-C in T allele F carriers and C/C M carriers with low CHO intake
Abellan et al. (2011) (17)	*ABCG1*	rs1044317 rs1893590 rs1378577 rs4148102 rs4148107	Epidemiologic and cohort studies	PUFA	1,941 M and F	Spanish	↑ TC and LDL-C in rs4148102-A/A carriers with ≥13.6 g/d PUFA intake *vs.* G allele carriers ↑ HDL-C in rs1044317-A allele carriers with ≥13.6 g/d PUFA intake *vs.* G/G carriers
Viturro et al. (2006) (18)	*ABCG5*	rs6720173	Cross-sectional	Dietary cholesterol and SFA	1,227 healthy children	Spanish	↑ TC and LDL-C in G allele M carriers with 14.3-34.1 g/d SFA intake *vs.* C/C M carriers
	ABCG8	rs6544718	Cross-sectional	Dietary cholesterol and SFA	1,227 healthy children	Spanish	↑ TC in C>T carriers with <300 mg/d cholesterol intake *vs.* C/C carriers ↑ LDL-C in C>T F carriers with <300 mg/d cholesterol intake *vs.* C/C carriers
Hwang et al. (2013) (19)	*ADIPOQ*	rs1501299	Prospective study	CHO	673 diabetic M and F	Korean	↓ LDL-C in T allele carriers with low (<55% en) CHO intake *vs.* G/G carriers ↑ HDL-C in T allele carriers with high (>65% en) CHO intake *vs.* G/G carriers
Phillips et al. (2011) (20)	*APOA1*	rs5069 rs5070 rs5081 rs670	Nested case-control (LIPGENE)	Total fat and FA	1,754 M and F	French	↑ LDL-C/HDL-C ratio in rs670-G/G carriers with >35% en fat intake *vs.* A allele carriers
Rudkowska et al. (2013) (21)	*APOA1*	rs670 rs5070	Survey (Qanuippitaa Nunavik Health)	Total fat and FA	553 M and F	Inuit	↑ TC in rs670-G>A carriers with high SFA intake ↑ LDL-C in rs670-G allele carriers with high total fat and SFA intake

Table 3.1 *Continued*

Reference	Gene	Polymorphism(s)	Study design	Diet	Population (n)	Ethnicity	Cholesterol response[1]
Rudkowska et al. (2013) (21)	*APOA1*	rs5070	Survey (Qanuippitaa Nunavik Health)	Total fat and FA	553 M and F	Inuit	↓ HDL-C in C/C carriers with high total fat and SFA intake
Hubacek et al. (2007) (22)	*APOA4*	rs675 rs5110	Cross-sectional (MONICA)	8-yr lower red meat, animal fat, and egg	133 M	Czech	↓ TC in haplotypes rs675-A/rs5110-C and rs675-T/rs5110-C with low intake *vs.* baseline
	APOA5	rs3135506	Cross-sectional (MONICA)	8-yr lower red meat, animal fat, and egg	133 M	Czech	↓ TC in G allele carriers with low intake *vs.* C/C carriers
Rudkowska et al. (2013) (21)	*APOB*	rs693	Survey (Qanuippitaa Nunavik Health)	Total fat and FA	553 M and F	Inuit	↑ TC and LDL-C in T allele carriers with high total fat and SFA intake *vs.* C/C carriers
Brown et al. (2003) (23)	*APOC3*	rs202197102	Cross-sectional	SFA	336 M and F	Costa-Rican	↑ TC and LDL-C in T allele carriers with high SFA *vs.* low SFA intake
Rudkowska et al. (2013) (21)	*APOC3*	rs5128	Survey (Qanuippitaa Nunavik Health)	Total fat and FA	553 M and F	Inuit	↑ LDL-C in C allele carriers with high total fat and SFA intake
Yang et al. (2007) (24)	*APOE*	E2, E3, E4	Case-control	Total fat and SFA	1,927 CVD case and 1,927 controls	Costa-Rican	↑ LDL-C in E2 and E4 carriers with high fat intake *vs.* non-carriers
Rudkowska et al. (2013) (21)	*APOE*	rs405509	Survey (Qanuippitaa Nunavik Health)	Total fat and FA	553 M and F	Inuit	↑ LDL-C in T/T carriers with high total fat intake ↓ HDL-C in T/T carriers with high total fat and SFA intake

Table 3.1 *Continued*

Reference	Gene	Polymorphism(s)	Study design	Diet	Population (n)	Ethnicity	Cholesterol response[1]
Rudkowska et al. (2013) (21)	*CETP*	rs5882	Survey (Qanuippitaa Nunavik Health)	Total fat and FA	553 M and F	Inuit	↑ TC in T/T carriers with high total fat intake
Hubacek et al. (2003) (25)	*CYP7A1*	rs3808607	Cross-sectional (MONICA)	8-yr lower red meat, animal fat, and egg	131 M	Czech	↓ TC in C/C carriers with low intake *vs.* A allele carriers
Chamberlain et al. (2009) (26)	*FABP2*	rs1799883	Prospective cohort (CARDIA)	20-yr total calorie and SFA	2,148 young M and F	Caucasian and African American	↓ HDL-C/TC ratio in T allele carriers with high (≥53.2 g/d) SFA intake *vs.* C/C carriers
Freitas et al. (2010) (27)	*HMGCR*	rs17238540	Prospective cohort (EPIC-Norfolk)	Total fat, FA, and fibre	23,011 healthy M and F	Mixed European	↓ TC and LDL-C in T/T carriers with >7.3% en PUFA and >22 g/d fibre intakes ↑ TC and LDL-C in T/T carriers with >14.3% en SFA intake ↑ HDL-C in T/T carriers with >36.3% en total fat, >14.3% en SFA, and >22 g/d fibre intakes
Nettleton et al. (2007) (28)	*LIPC*	rs1800588	Prospective cohort (ARIC)	Total fat and FA	11,806 M and F	Caucasian and African American	↓ HDL-C in C/C African American F carriers with low fat intake *vs.* T allele carriers ↑ HDL-C in T/T African American M carriers with high fat intake *vs.* C allele carriers
Riestra et al. (2009) (29)	*LIPC*	rs1800588	Cross-sectional	Total fat and FA	1,260 healthy children	Spanish	↑ HDL-C in T allele M carriers with higher total and major types of fat intake *vs.* C/C carriers

Table 3.1 *Continued*

Reference	Gene	Polymorphism(s)	Study design	Diet	Population (n)	Ethnicity	Cholesterol response[1]
Tai et al. (2003) (30)	*LIPC*	rs1800588	Survey (Singapore National Health)	Total fat	2,170 M and F	Chinese, Malays, and Indian	↑ HDL-C in T/T Indian carriers with <30% en and lower with ≥30% en fat intake *vs.* C/C carriers
Nettleton et al. (2007) (28)	*LPL*	rs328	Prospective cohort (ARIC)	Total fat and FA	11,645 M and F	Caucasian and African American	↑ HDL-C in Caucasian C allele carriers and lower in G/G carriers with high total fat, SFA, and MUFA intakes
Kim et al. (2013) (31)	*LPL*	rs285 (PvuII) rs320 (HindIII) haplotypes	Cross-sectional (KMSRI-Seoul cohort)	CHO and fat	269 controls and 280 MetS patients	Korean	↓ HDL-C in P2H2 carriers with high CHO *vs*. P1H1 carriers with low CHO intake
Baik et al. (2013) (32)	*LPL*	rs10503669	Two cohorts within the Korean Genome and Epidemiology Study	USFA and alcohol	5,314 M and F	Korean	↑ HDL-C in T allele carriers with higher USFA intake *vs*. G/G carriers
Robitaille et al. (2007) (33)	*LXRA*	rs12221497 rs61896015 rs3758674	Cross-sectional	Dietary cholesterol	35 hyperlipidemic M	French Canadian	↑ TC and LDL-C in rs12221497-A, rs61896015-A, and rs3758674-C allele carriers with >290 mg/d cholesterol intake *vs.* G/G, C/C, and T/T carriers, respectively
Fontaine-Bisson et al. (2009) (34)	*NFKB*	rs28362491	Cross-sectional	Total fat and FA	593 diabetes-free M and F, and 103 diet-treated diabetes F	Canadian	↑ HDL-C in Ins/Ins carriers and ↓ in Del/Del carriers with high PUFA intake

Table 3.1 *Continued*

Reference	Gene	Polymorphism(s)	Study design	Diet	Population (n)	Ethnicity	Cholesterol response[1]
Volcik et al. (2008) (35)	*PPARA*	rs6008259 rs3892755	Prospective cohort (ARIC)	n-6 and n-3 PUFA	13,614 M and F	Caucasian and African American	↓ TC and LDL-C in rs6008259-A/A Caucasian carriers with >7.99 g/d n-6 intake *vs.* G allele carriers ↓ TC and LDL-C in rs3892755-T/T African American carriers with >0.32 g/d n-3 intake *vs.* C allele carriers
Robitaille et al. (2007) (36)	*PPARD*	rs2016520	Cross-sectional	Total fat and en intake	340 healthy M and F	French Canadian	↓ TC/HDL-C ratio in C allele carriers with <34.4% en fat intake *vs.* T/T carriers ↑ HDL-C in C allele carriers with <34.4% en fat intake *vs.* T/T carriers
Memisoglu et al. (2003) (37)	*PPARG*	rs1801282	Case-control (Nurses' Cohort)	Total fat	656 F	Mostly Caucasian	↓ TC in C/C carriers with high fat intake ↓ HDL-C in C/C carriers and ↑ in G allele carriers with high fat intake
Rudkowska et al. (2013) (21)	*PPARG2*	rs10856710	Survey (Qanuippitaa Nunavik Health)	Total fat and FA	553 M and F	Inuit	↑ TC and LDL-C in C allele carriers with high total fat and SFA intakes
Fontaine-Bisson et al. (2007) (38)	*TNFA*	rs361525 rs1800629	Cross-sectional	Total fat and FA	109 diabetic M and F	Canadian	↑ HDL-C in rs361525-A allele carriers with high PUFA intake. Opposite trend in rs361525-G/G carriers and rs1800629-A allele carriers
Joffe et al. (2010) (39)	*TNFA*	rs1800629	Cross-sectional	Total fat and FA	105 normal-weight and 118 obese F	black South African	↓ TC/HDL-C ratio in A allele carriers with increased (0-0.5% en) n-3 ALA intake ↑ LDL-C in A allele carriers with increased (0-20% en) PUFA intake

Table 3.1 *Continued*

Reference	Gene	Polymorphism(s)	Study design	Diet	Population (n)	Ethnicity	Cholesterol response[1]
Joffe et al. (2011) (40)	*TNFA*	rs1800629	Cross-sectional	Total fat and FA	88 normal-weight and 60 obese F	white South African	↓ TC in G/G carriers and ↑ in A allele carriers with high SFA intake
Joffe et al. (2012) (41)	*TNFA*	rs361525	Cross-sectional	Total fat and FA	169 normal-weight and 182 obese F	black and white South African	↑ TC/HDL-C ratio in G>A carriers and ↓ in G/G carriers with increased n-6:n-3 intakes ↓ HDL-C in G>A and ↑ in G/G carriers with increased n-6:n-3 intakes

[1] Direction of effect on biomarkers of circulating cholesterol responses (↑ higher; ↓ lower). %, percentage; ABC, ATP-binding cassette sub-family; *ADIPOQ*, adiponectin; ALA, alpha linolenic acid; APO, apolipoprotein; ARIC, Atherosclerosis Risk in Communities; CARDIA, Coronary Artery Risk Development in Young Adults; *CETP*, cholesteryl ester transfer protein; CHO, carbohydrate; *CYP7A1*, cholesterol 7-alpha-hydroxylase; d, day; en, energy; EPA, eicosapentaenoic acid; EPIC, European Prospective Investigation into Cancer and Nutrition; F, females; FA, fatty acids; g, gram; HDL-C, high-density lipoprotein cholesterol; *HMGCR*, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; kcal, kilocalories; KMSRI, Korean Metabolic Syndrome Research Initiatives; LDL-C, low-density lipoprotein cholesterol; *LIPC*, hepatic lipase; *LPL*, lipoprotein lipase; *LXRA*, liver X receptor alpha; M, males; MetS, metabolic syndrome; mg, milligram; MONICA, Multinational MONItoring of trends and determinants in CArdiovascular disease; MUFA, monounsaturated fatty acids; *NFKB*, nuclear factor kappa B; *PPARA*, peroxisome proliferator-activated receptor alpha; *-D*, delta; *-G*, gamma; preM, premenopausal; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; SNP, single nucleotide polymorphism; TC, total cholesterol; *TNFA*, tumor necrosis factor alpha; USFA, unsaturated fatty acids; yr, year.

Table 3.2 Summary of dietary intervention studies with significant impact of gene-diet interactions on circulating cholesterol levels

Reference	Gene	Polymorphism(s)	Study design	Diet	Population (n)	Ethnicity	Cholesterol response[1]
Guevara-Cruz et al. (2010) (42)	*ABCA1*	rs9282541 rs2230806	Prospective cohort	4-wk LSF followed by 8-wk LSF + SSF	43 hyperlipidemic M and F	Mexican	↑ HDL-C in rs9282541-C>T carriers with LSF + SSF intake *vs.* C/C carriers
Zhao et al. (2008) (43)	*ABCG5/G8*	*ABCG5* rs6720173 *ABCG8* rs11887534 rs4148217 rs6544718	Crossover	4-wk fat spread with or without (2 g/d) PS	82 hypercholesterolemic M	Caucasian	↓ TC and LDL-C in *ABCG8* rs4148217-A allele carriers with PS intake and high *vs.* low basal plasma PS levels
Gomez et al. (2010) (44)	*APOA1*	rs1799837	Crossover	4-wk SFA followed by low-fat/high-CHO or MUFA	97 healthy M and F	Spanish	↑ LDL-C in G/G carriers with CHO and ↓ LDL-C with MUFA intake *vs.* A allele carriers
Zhang et al. (2012) (45)	*APOA5*	rs964184	Weight-loss intervention (Pounds Lost Trial)	2-yr of 4 diets differing in % en from fat, protein, and CHO	734 overweight or obese M and F	Mostly Caucasian	↓ TC and LDL-C in G allele carriers with low-fat (20% en) intake *vs.* C/C carriers ↑ HDL-C in G allele carriers with high-fat (40% en) intake *vs.* C/C carriers
Jemaa et al. (2004) (46)	*APOB*	rs17240441 (Ins/Del)	Low-calorie intervention	10-wk of 25% reduction in en intake	231 overweight M and F	French Caucasian	↓ TC and LDL-C in Del/Del carriers *vs.* Ins allele carriers

Table 3.2 *Continued*

Reference	Gene	Polymorphism(s)	Study design	Diet	Population (n)	Ethnicity	Cholesterol response[1]
Song et al. (2011) (47)	*APOC3*	rs5128 (SstI; S1 is the major allele)	Intervention	7-d washout followed by 6-d high (70% en) CHO	56 healthy university students	Chinese	↑ TG/HDL-C ratio in S2 F carriers with high CHO intake *vs.* S1S1 carriers
Sanchez-Muniz et al. (2009) (48)	*APOE*	E2, E3, E4	Parallel	5-wk fat spread with or without (1.1 or 2.2 g/d) PS	217 hypercholesterolemic M and F	Spanish	↓ TC, LDL-C, TC/HDL-C, and LDL-C/HDL-C ratios in E2 and E3 carriers with PS *vs.* placebo intake
Moreno et al. (2009) (49)	*APOE*	E2, E3, E4	Crossover	4-wk SFA followed by low-fat/high-CHO or MUFA	84 healthy young M and F	Spanish	↓ HDL-C in E3/2 F carriers with CHO or MUFA intake *vs.* E3/3 and E4/3 carriers
Tucker et al. (2010) (50)	*APOE*	E2, E3, E4	Crossover	6-wk whole-grain wheat or white bread (2/d)	28 M and F (14 NGI and 14 HGI)	Not specified	↑ LDL-C in E3/E3 NGI carriers with whole grain *vs.* white bread intake ↑ TG/HDL-C ratio in E3/3 HGI carriers with whole grain *vs.* white bread intake
Moreno et al. (2004) (51)	*APOE*	rs405509	Crossover	4-wk SFA followed by high-CHO or MUFA	55 healthy *APOE* E3/E3 M	Spanish	↑ LDL-C in T allele carriers with SFA intake *vs.* G/G carriers

Table 3.2 *Continued*

Reference	Gene	Polymorphism(s)	Study design	Diet	Population (n)	Ethnicity	Cholesterol response[1]
Madden et al. (2008) (52)	*CD36*	rs1527483 27645del/ins rs1049673 rs1761667 rs1984112	Intervention	12-wk habitual diet + 6 x 1 g/d fish oil capsules (~1 g/d EPA)	111 healthy M	Caucasian	↑ LDL-C in rs1761667- and rs1984112-A/A carriers with EPA *vs.* baseline ↑ HDL-C in rs1761667-A/A and A>G carriers, rs1984112-A/A carriers, rs1527483-G/G carriers, and rs1049673-C/C and C>G carriers with EPA *vs.* baseline
Goyenechea et al. (2008) (53)	*CD36*	rs2151916	Intervention	8-wk low calorie	183 obese M and F	Spanish and British	↓ TC in C allele carriers *vs.* baseline
Du et al. (2010) (54)	*CETP*	rs708272 (TaqIB; B1 = presence of restriction site)	Intervention	7-d washout followed by 6-d high (70% en) CHO	56 healthy university students	Chinese	↑ HDL-C in T (B2) allele carriers with high CHO *vs.* washout diet
De Castro-Oros et al. (2010) (55)	*CYP7A1*	rs3808607	Parallel	4-8 wk Mediterranean diet with or without 2 and 3.2 g/d PS esters	67 normo- and hypercholesterolemic M and F	Spanish	↓ TC in C allele carriers with PS ester intake *vs.* A/A carriers
Kovar et al. (2004) (56)	*CYP7A1*	rs3808607	Cross-over	3-wk high-fat (40% en) or low-fat (22% en) intake	11 healthy M	Czech	↑ TC and LDL-C in C/C carriers after high- *vs*. low-fat intakes

Table 3.2 *Continued*

Reference	Gene	Polymorphism(s)	Study design	Diet	Population (n)	Ethnicity	Cholesterol response[1]
Hofman et al. (2004) (57)	*CYP7A1*	rs3808607	Pooled data from 8 trials with dietary cholesterol	3-4 wk cholesterol-rich diet (742 ± 114 mg/d) intake	144 normolipidemic M and F	Mainly Dutch	↑ TC in C allele carriers with cholesterol intake *vs.* A/A carriers ↑ HDL-C in C allele carriers with cholesterol intake *vs.* A/A carriers
de Luis et al. (2008) (58)	*FABP2*	rs1799883	Parallel	8-wk hypocaloric low-fat and low-CHO	204 obese M and F	Spanish	↓ TC in C/C carriers with both diets *vs.* baseline
Pishva et al. (2010) (59)	*FABP2*	rs1799883	Intervention	8-wk habitual diets with (2 g/d) EPA	46 hypertriglyceridemic M and F	Iranain	↑ HDL-C in T allele carriers with EPA intake from baseline *vs.* C/C carriers
Rubin et al. (2012) (60)	*FABP2*	rs2282688 (haplotype A) rs1799883 (haplotype B)	Intervention	2-wk dietary MCT and LCT	82 healthy M	German	↑ HDL-C in haplotype A carriers with MCT intake
Du et al. (2010) (54)	*LDLR*	rs285 (Pvu II; P1 = absence of restriction site)	Intervention	7-d washout followed by 6-d high (70% en) CHO	56 healthy university students	Chinese	↑ HDL-C in T/T (P1P1) carriers with high CHO *vs.* washout diet
Huang et al. (2011) (61)	*LPL*	rs328	Intervention	7-d washout followed by 6-d high (70% en) CHO	56 healthy university students	Chinese	↓ TC and LDL-C in C/C F carriers with high CHO *vs.* washout diet

Table 3.2 *Continued*

Reference	Gene	Polymorphism(s)	Study design	Diet	Population (n)	Ethnicity	Cholesterol response[1]
Huang et al. (2011) (61)	*LPL*	rs320	Intervention	7-d washout followed by 6-d high (70% en) CHO	56 healthy university students	Chinese	↓ TC in T/T F carriers with high CHO *vs.* washout diet ↑ HDL-C in rs320-G allele M carriers with high CHO *vs.* washout diet
Zhao et al. (2008) (43)	*NPC1L1*	rs2072183 rs10264715	Crossover	4-wk fat spread with or without (2.0 g/d) PS	82 hypercholesterolemic M	Caucasian	↓ TC and LDL-C in rs2072183-G/rs10264715-A haplotype mutant allele carriers with PS intake *vs.* wild-type allele carriers
Alsaleh et al. (2011) (62)	*PPARG*	rs1801282	Parallel (RISCK Study)	4-wk high-SFA (HS; 18% en)	367 M and F	Caucasian	↑ TC and LDL-C in G allele carriers with low (≤0.33) PUFA:SFA ratio intake *vs.* C/C carriers
Hamada et al. (2009) (63)	*UCP1*	rs1800592	Intervention	8-wk low calorie	32 obese F	Japanese	↓ HDL-C in A/A carriers from baseline *vs.* G allele carriers
Luis et al. (2009) (64)	*UCP3*	rs1800849	Parallel	8-wk hypocaloric low-fat and low-CHO	131 obese M and F	Spanish	↓ TC in C/C carriers with low CHO intake *vs.* baseline

[1] Direction of effect on biomarkers of circulating cholesterol responses (↑ higher; ↓ lower). %, percentage; ABC, ATP-binding cassette sub-family; APO, apolipoprotein; *CD36*, cluster of differentiation 36; *CETP*, cholesteryl ester transfer protein; CHO, carbohydrate; *CYP7A1*, cholesterol 7-alpha-hydroxylase; d, day; en, energy; EPA, eicosapentaenoic acid; F, females; *FABP2*, fatty acid binding protein; g, gram; HDL-C, high-density lipoprotein cholesterol; HGI; hyperglycemic/hyperinsulinemic; HS, high SFA; LCT, long chain triglycerides; LDL-C, , low-density lipoprotein cholesterol; *LDLR*, LDL receptor; *LPL*, lipoprotein lipase; LSF, low saturated fat; M, males; MCT, medium chain triglycerides; MUFA, monounsaturated fatty acids; NGI, normoglycemic/normoinsulinemic; *NPC1L1*, Niemann-Pick C1 Like 1; *PPARG*, peroxisome proliferator-activated receptor gamma; PS, plant sterol; RISCK, Reading, Imperial, Surrey, Cambridge, King's; SFA, saturated fatty acids; SSF, soy/soluble fibre; TC, total cholesterol; TG, triglycerides; *UCP*, uncoupling protein; wk, week; yr, year.

impact of the study's minor allele T was more recently confirmed in a cross-sectional research that involved a nationwide population sample of 3,591 adults in Mexico (16). There, serum HDL-C levels were negatively correlated with dietary carbohydrate (CHO) intake, especially among the rs9282541-T allele female carriers (β coefficient = −0.16%) and the C/C male homozygotes (β coefficient = −0.13%). This correlation was greater in the T allele premenopausal female carriers (β coefficient = −0.30%) but not postmenopausal women (16). Accordingly, it appears that dietary portfolios of lower saturated fatty acid (SFA) and/or CHO intakes may be of special benefits to individuals with certain genotypes in the *ABCA1* SNP rs9282541.

3.3.2 ABCG1 polymorphisms modulate circulating cholesterol responses to high polyunsaturated fat intake

ATP-binding cassette sub-family G1 (ABCG1) is a sterol transporter involved in cholesterol and phospholipids efflux from macrophages towards HDL particles (67).

Individuals carrying the *ABCG1* SNP rs1044317-A allele showed higher plasma HDL-C levels compared to the G/G homozygotes (1.4 *vs.* 1.3 mmol/L, respectively) on a diet high (≥13.6 g/day) in polyunsaturated fatty acids (PUFA) (17). On this high PUFA diet too, *ABCG1* SNP rs448102-A/A homozygotes showed 22.3% and 35.5% higher total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels, respectively, compared to the study's major allele G carriers. The authors of this epidemiological study based on dietary data from 24-hr recalls, food records, and food frequency questionnaires of 1,941 individuals, thus, suggested an *ABCG1* genotype-diet interaction only when high

amounts of PUFA are consumed, where carriers of *ABCG1* SNP rs1044317-A and SNP rs448102-G alleles may show reduced CVD risks.

3.3.3 ABCG5/G8 polymorphisms modulate circulating TC and LDL-C responses to plant sterol, low saturated fat, and low dietary cholesterol intakes

ATP-binding cassette sub-family G5 (ABCG5) is a half-transporter that, jointly with ABCG8, is responsible for phytosterol and cholesterol excretion from enterocytes into the intestinal lumen, and from hepatocytes into the bile. Loss-of-function mutations in *ABCG5/G8* have been determined as causes for phytosterolemia/sitosterolemia, a rare recessive condition featuring abnormally elevated serum phytosterol levels associated with premature atherosclerosis (68).

After 4 weeks of plant sterol (PS) consumption (2 g/day), only hypercholesterolemic men carrying the *ABCG8* SNP rs4148217 (1289C>A) A allele showed 2.5- and 3.9-fold greater reductions in serum TC and LDL-C levels, respectively, when they had high plasma PS levels at baseline (43). Independently, Torres et al. (69) reported no association between *ABCG5* SNP rs6720173 or *ABCG8* SNP rs11887534 and cholesterol responses to daily intake of soy/soluble fibre with 6% of total energy (6% en) coming from a SFA background diet for 8 weeks. Amongst Spanish children with a low SFA diet intake (14.3-34.1 g/day), only male carriers of the *ABCG5* SNP rs6720173-G (604Glu) allele showed higher plasma TC (4.9 *vs.* 4.5 mmol/L, respectively) and LDL-C (3.1 *vs.* 2.6 mmol/L, respectively) levels compared to the C/C (604Gln) homozygotes (18). In the same cross-sectional study, *ABCG8* SNP rs6544718-C>T (Ala632Val) heterozygotes had higher plasma TC levels (4.8 and 5.1 mmol/L for males and females, respectively)

compared to the C/C homozygotes (4.6 mmol/L) only when cholesterol intake was less than 300 mg/day. Also with this dietary cholesterol intake, the *ABCG8* SNP rs6544718-C>T female heterozygotes had higher LDL-C levels (3.2 *vs.* 2.8 mmol/L, respectively) compared to the C/C homozygotes. These data accordingly suggest that, among Spanish children, carriers of the *ABCG8* SNP rs6544718 minor allele T may not benefit from restricting their intakes of dietary cholesterol. Whether such observation is found in adults is, to our knowledge, yet to be known.

3.3.4 ADIPOQ polymorphisms modulate circulating HDL-C and LDL-C responses to various carbohydrate intakes

Adiponectin is a protein/hormone secreted exclusively by adipose tissues and is involved in regulating fatty acid (FA) and glucose metabolism. It also performs complementary functions with leptin, another adipose-derived hormone involved in energy metabolism. Circulating levels of adiponectin have been shown to be inversely linked with body fat mass in adults, as well as to risk for type 2 diabetes and atherosclerosis (70).

A prospective cohort involving 673 diabetic individuals in South Korea reported slightly higher serum HDL-C levels (1.2 mmol/L) in carriers of the *ADIPOQ* SNP rs1501299-T allele in comparison to the G/G homozygotes (1.1 mmol/L) only with higher (>65% en) CHO intake (19). With <55% en intake from CHO, the rs1501299-T allele carriers showed lower plasma LDL-C levels than the G/G homozygotes (2.7 *vs.* 3.0 mmol/L, respectively). In contrast, no such associations were previously reported between certain *ADIPOQ* SNPs and SFA-, CHO-, and monounsaturated fatty acid (MUFA)-rich diets (71) or with total fat, SFA, MUFA, or PUFA intakes (72).

3.3.5 APOA1 polymorphisms modulate circulating LDL-C responses to high carbohydrate, monounsaturated fat, and saturate fat intakes

Apolipoprotein A-I is the major protein component of HDL particles, which are involved in reverse cholesterol transport to the liver and are associated with protection against CVD (73). apo A-1 also acts as a cofactor for lecithin cholesterol acyltransferase, an enzyme that converts free cholesterol into cholesteryl ester in a process that eventually leads to synthesis of new HDL particles (74). *APOA1* gene, of which hepatocytes are the main site of expression, is part of the *APOA1/C3/A4/A5* gene cluster.

In a dietary crossover design involving 97 healthy Spanish individuals, higher plasma LDL-C levels (2.2 mmol/L) were reported in the *APOA1* SNP rs1799837 (-76G>A) G/G homozygotes after intake of a higher CHO diet (28% total fat, 57% CHO) compared with the A allele carriers (2.0 mmol/L) (44). However, lower LDL-C levels (2.2 *vs.* 2.4 mmol/L) were reported in the same individuals after a MUFA diet intake (22% MUFA, 38% total fat, 47% CHO). Carriers of the SNP rs1799837-G/G genotype also showed lower LDL-C levels upon changing from a SFA diet (20% SFA, 38% total fat, 47% CHO) to CHO (0.36 mmol/L) or MUFA (0.40 mmol/L) diets, whereas the A-allele carriers had higher LDL-C levels after switching from the CHO to SFA (0.6 mmol/L) or MUFA (0.4 mmol/L) diets (44). Observational data from the nested-case control LIPGENE-SU.VI.MAX study (20), moreover, suggested a higher serum LDL/HDL ratio (2.6 *vs.* 2.5, respectively) for *APOA1* SNP rs670-G/G homozygotes relative to the A-allele carriers on a high-fat (>35% en) diet intake. The rs670-G/G homozygotes also showed higher HDL-C levels at baseline relative to the A/G + A/A genotype carriers (1.6

vs. 1.5 mmol/L, respectively). With a low-fat diet intake, however, no differences in cholesterol levels were observed (20). This role of *APOA1* SNPs rs670, as well as of SNP rs5070 in modifying plasma cholesterol level responses to dietary total fat and/or SFA intakes was more recently confirmed (21). On the other hand, no significant effects on circulating cholesterol levels were seen in two earlier studies that examined gene-diet interactions with SNP rs670 (69), or with SNPs rs670 and rs5069 (22).

3.3.6 APOA4 haplotypes modulate circulating TC responses to long-term low fat intake

The *APOA4* gene is mainly expressed in the small intestine where it encodes apolipoprotein A-IV, a component of chylomicron particles and an activator of lecithin cholesterol acyltransferase *in vitro*. Synthesis and secretion of apolipoprotein A-IV are increased by intestinal fat absorption (75).

Czech males carrying the common *APOA4* haplotypes rs675-A and rs5110-C, and rs675-T and rs5110-C, alleles showed reduced plasma TC levels (5.5 *vs.* 6.3 mmol/L, respectively) after versus before a long-term (eight years) adherence to a diet lower in red meat and animal fats but higher in fruits and vegetables (22). Authors of the 2007 study defined these variants by their encoding amino acid changes as 347Thr/360Gln and 347Ser/360Gln, however the dbSNP lists the changes by SNP rs675–G>T>A variants as amino acid 367Ala>Ser>Thr, and the changes for rs5110–C>A as amino acid 380Gln>His. These *APOA4* SNPs were not associated with impacts on circulating cholesterol responses in two other dietary studies, however (44,76).

3.3.7 APOA5 polymorphisms modulate circulating cholesterol responses to long-term low and high fat intakes

Apolipoprotein A-V is a component of lipoprotein fractions and a mediator of circulating triglyceride (TG) levels, which are a major biomarker for cardiovascular health (77). Certain forms of hypertriglyceridemia and hyperlipoproteinemia are related to variations in *APOA5* (78).

Among Czech males, the *APOA5* SNP rs3135506-G (Try19) allele carriers showed a 20% decrease in plasma TC levels (from 6.5 to 5.1 mmol/L) in response to a drop in the consumption of red meat, animal fat, and eggs over 8 years, when compared to the C/C (Ser19) homozygotes, who only showed an 8% decrease (from 6.1 in 1988 to 5.6 mmol/L in 1996) (22). On the other hand, in a 2-year weight-loss dietary intervention involving four diets that varied in energy intake from fat, protein, and CHO, the *APOA5* SNP rs964184-G allele carriers showed greater reductions in plasma TC and LDL-C levels (β coefficients = −9.3 and −7.5, respectively) with low-fat (20% en) intake, and greater increase in HDL-C levels (β coefficient = +1.7) with high-fat (40% en) intake relative to the C/C homozygotes (45). No *APOA5* genotype x dietary fat interactions were, however, previously shown in individuals from the Framingham Heart Study (79).

3.3.8 APOB polymorphisms modulate circulating LDL-C and TC responses to low-calorie diet, and high total and saturated fat intakes

Apolipoprotein B, mainly synthesized by the liver and small intestine, is the main protein component of the circulating TG-rich chylomicrons and LDL particles. Some evidence

presently suggests that circulating levels of apoB-48 and apoB-100, the two main isoforms of apolipoprotein B, may be better indicators of CVD risk than the traditional TC and LDL-C levels (80).

Among French Canadians, carriers of the *APOB* SNP rs17240441 (also known as Ins/Del) Del/Del genotype showed greater reduction (−16.8%) in plasma LDL-C levels on a 10-week low-calorie diet (LCD; 25% lower en) intake in comparison to the Ins/Del (−4.7%) and Ins/Ins (+0.9%) carriers (46). Circulating cholesterol responses to the *APOB* gene-diet interactions were also reported in Inuit populations, where *APOB* SNP rs693-T/T homozygotes showed higher plasma TC and LDL-C levels with higher intakes of total fat (β coefficients = 0.0096 for TC and 0.0089 for LDL-C) and SFA (β coefficients = 0.0246 for TC and 0.0240 for LDL-C) compared to the C/C homozygotes (21). Neither of two other studies with *APOB*, however, showed significant responses of cholesterol metabolism to interactions between *APOB* genotypes and changes in dietary fat intake (20,81). For instance, although the presence of *APOB* SNP rs512535 was shown to modulate the risk of metabolic syndrome when in interaction with high fat (>35% en) or high MUFA (>14% en) intakes, no differences in lipid profile were observed (20).

3.3.9 APOC3 polymorphisms modulate circulating cholesterol responses to high carbohydrate/low fat diet, and high total and saturated fat intakes

APOC3 gene is on a cluster with the *APOA1* and *APOA4* genes on chromosome 11, where it encodes apolipoprotein C-III, which inhibits lipoprotein lipase (LPL) and hepatic lipase activities. Increases in circulating apolipoprotein C-III levels have been associated with hypertriglyceridemia (82).

Chinese female, but not male, carriers of the *APOC3* SNP rs5128-G allele, which is often called *APOC3 SstI*-2 allele, as in Song et al. (47) showed an elevated serum TG/HDL-C ratio after the intake of a high-CHO/low-fat diet (HC/LF; 70% en CHO, 15% fat, 15% protein) for 6 days in comparison to baseline values (1.6 *vs.* 1.4, respectively). There, the HC/LF diet also lowered TC/HDL-C and LDL-C/HDL-C ratios regardless of the gender and genotype (47). Higher intakes of SFA were associated with 13% and 20% higher plasma TC and LDL-C levels, respectively, in comparison to a habitually low SFA intake in Costa Rican homozygotes for the promoter SNP rs202197102-T allele (23). Among Inuit individuals who carry the *APOC3* rs5128-C allele higher plasma LDL-C levels were associated with higher total fat (β coefficients = 0.0054 and 0.0048 for C/C and C/G carriers, respectively) and SFA (β coefficients = 0.0154 and 0.0128 for C/C and C/G carriers, respectively) intakes than among the G/G homozygotes (21). In other studies, however, no apparent gene-diet interactions were found between *APOC3* SNPs rs5128 or rs2854117 and cholesterol responses to a drop in animal fat intake over 8 years (22) or a 4-week high cholesterol diet (~640 mg/day) (76).

3.3.10 APOE genotypes modulate circulating cholesterol responses to plant sterol, high carbohydrate, monounsaturated, and saturated fat intakes

Apolipoprotein E is the chief protein on chylomicrons and very low-density lipoproteins, and their remnant particles, where it promotes the uptake of TG and cholesterol from the circulation into peripheral tissues. In gene-diet and lipid metabolism studies, *APOE* is the most widely studied gene, with 3 allelic variants (ε2, ε3, and ε4) coding for E2, E3, and E4 isoforms and eventually yielding 6 possible genotypes (E2/2, E3/2, E4/2, E3/3, E3/4,

and E4/4). Notably, a sound body of evidence suggests that carriers of the E4 allele are at higher risk of developing CVD (83).

Hypercholesterolemic carriers of *APOE* E2 and E3 alleles showed lower serum TC (% change = −10.2 for E2 and −3.9 for E3) and LDL-C (% change = −12.7 for E2 and −5.5 for E3) levels, as well as lower TC/HDL-C (% change = −10.3 for E2 and −5.4 for E3) and LDL-C/HDL-C (% change = −12.7 for E2 and −8.5 for E3) ratios, after consuming 1.1 or 2.2 g/day PS esters versus low PS placebo diet (48). These data agree with older findings by Song et al. (83) suggesting a little value for *APOE* E4 carriers with PS intake. Further, greater decreases in plasma HDL-C levels were reported in female carriers of E3/E2 (−16 and −13%) versus those of E3/E3 (−8 and −7%) and E4/E3 (−4 and −1%) genotypes after switching from a SFA-rich diet (20% SFA, 38% fat) to CHO- (55% CHO, 30% fat, <10% SFA) or MUFA-rich (22% MUFA, 38% fat) diets (49). Moreover, normoglycemic or normoinsulinemic carriers of E3/E3 genotype showed higher serum LDL-C levels (3.4 *vs.* 3.1 mmol/L, respectively) in response to a 6-week intake of whole grain wheat sourdough compared to white bread. Carriers of E3/3 genotype who were hyperglycemic or hyperinsulinemic, on the other hand, showed higher TG levels (2.3 *vs.*1.8 mmol/L) and TG/HDL-C ratio (2.3 *vs.* 1.7) following the consumption of the whole grain bread (50). Compared to these dietary interventions, data from case-control studies are less clear. For example, among 1,927 CVD case and 1,927 population-based control Costa-Rican individuals, Yang et al. (24) reported higher plasma LDL-C levels among carriers of the *APOE* E2 (+17%) and E4 (+14%) in comparison to non-carriers (+6%) on a high fat diet intake. The observation thus supported the hypothesis that the E2 and E4 genotypes are associated with higher risk of CVD with a high SFA diet intake.

Additionally, on a high total fat intake, Inuit homozygotes of the *APOE* SNP rs405509 (-219G>T) T allele showed higher plasma LDL-C levels (β coefficient = 0.0034) than G/G homozygotes, and lower HDL-C (β coefficient = −0.0010) levels than the G>T heterozygotes (21). These observations resembled others reported previously by Moreno et al. (51), where in a randomized crossover design carriers of the *APOE* SNP rs405509 (-219G>T) T allele had higher plasma LDL-C levels (% change = 2.8 and 2.7 mmol/L for T/T homozygotes and T>G heterozygotes, respectively) after 4-week of SFA-rich diet (20% total en) than did the G/G homozygotes (% change = 2.2 mmol/L). Also, carriers of the rs405509-T allele showed a greater reduction in LDL-C levels when they switched to a 55% en CHO diet intake in comparison to the G/G homozygotes (T/T = −21%, G>T = −17%, G/G = −5%). Contrary to these data, other studies reported no association between *APOE* genotypes and circulating cholesterol responses to SFA- or PUFA-rich diets (84,85), low saturated fat + soy/soluble fibre diet (69), 2 g/day PS diet (86), total fat intake (87), low-fat, high-fat high-SFA, and high-fat high-SFA + docosahexaenoic acid diets (88), or polyphenols intake (89), whereas some research suggested an association in E2/4 carriers alone, which represented only a small percentage (3%) of the population under study (90).

3.3.11 CD36 polymorphisms modulate circulating cholesterol responses to fish oil and low-calorie diet intakes

Cluster of differentiation 36 (CD36) is a membrane glycoprotein mainly found on the surface of adipocytes, enterocytes, and hepatocytes, where it is involved in regulating FA and glucose transport (91).

In a 12-week intervention of fish oil capsules (6 x 1 g/day) providing ~1.0 g eicosapentaenoic acid and ~0.7 g docosahexaenoic acid daily, only homozygotes for the *CD36* genes SNPs rs1761667-A and rs1984112-A alleles showed higher plasma LDL-C levels (0.3 and 0.2 mmol/L, respectively) than baseline (52). Fish oil intake also raised HDL-C levels in the rs1761667-A/A homozygotes and A>G heterozygotes by 0.07 and 0.05 mmol/L, respectively, rs1984112-A/A homozygotes by 0.08 mmol/L, rs1527483-G/G homozygotes by 0.04 mmol/L, and rs1049673-C/C homozygotes and C>G heterozygotes by 0.05 and 0.06 mmol/L, respectively, in comparison to baseline values. In another study of an 8-week long LCD, followed by a weight-maintenance period (53), only carriers of the *CD36* SNP rs2151916 (-22674T>C) C allele showed lower serum TC levels in comparison to their baseline values. Moreover, rs2151916-C allele obese/overweight carriers showed lower levels of TC and LDL-C, but higher HDL-C, six months after the LCD.

3.3.12 CETP polymorphisms modulate circulating HDL-C and TC responses to high carbohydrate/low fat diet and high total fat intakes

Cholesteryl ester transfer protein (CETP) facilitates cholesteryl ester and TG transport from HDL particles to other lipoproteins. CETP is also a key contributor to the process of reverse cholesterol transport from peripheral cells to the liver (92).

Healthy young Chinese adult homozygotes for the *CETP* SNP rs708272-T allele (described by the study authors as *TaqIB* B2 carriers) responded to a HC/LF diet (70% en CHO, 15% protein, 15% fat) with higher serum HDL-C levels than when on a regular (washout) diet of 54% en from CHO, 15% from protein, and 31% from fat (1.5 *vs.* 1.4

mmol/L, respectively) (54). In the same study, only male carriers of the SNP rs708272-T allele showed increased HDL-C levels (1.4 *vs.* 1.2 mmol/L, respectively) after the washout diet in comparison to the C/C homozygotes. On a high total fat intake in the Qanuippitaa Nunavik Health Survey, conducted among 553 Inuit in 2004, higher plasma TC levels were observed in the homozygote carriers of the *CETP* SNP rs5882-T allele in comparison to the C>T heterozygotes (β coefficients = 0.0024 *vs.* −0.0029, respectively) (21). No significant associations were, however, detected between *CETP* SNP rs708272 and circulating lipid profile in a crossover trial of 35 individuals who consumed a low-fat background diet with SFA- or PUFA-rich diets (84), or in a prospective cohort data set for total fat and FA intakes measured by a 66-item food frequency questionnaire in 11,559 Caucasians and African Americans (28).

3.3.13 CYP7A1 polymorphisms modulate circulating TC and LDL-C responses to plant sterol, long-term low fat, and high-fat diet intakes

Cholesterol 7-alpha-hydroxylase (CYP7A1) is a rate-limiting enzyme for bile acid synthesis in the liver, which is the principal mechanism for cholesterol removal from the body (93).

Carriers of the *CYP7A1* SNP rs3808607 (-204A>C) C allele showed greater reductions in plasma TC levels compared with the A/A homozygotes (−0.43 *vs.* −0.14 mmol/L) with the intake of 2.0 and 3.2 g/day PS esters, in parallel arm studies employing a Mediterranean-style background diet (55). This seemingly larger response of individuals carrying the C allele to dietary intakes was previously examined among 131 Czech males whom food habits had changed over 8 years towards lower consumption of red meat and

animal fat, and higher consumption of fruits and vegetables. There, homozygote carriers of the SNP rs3808607-C allele in particular showed a greater reduction in plasma TC levels (−1.3 mmol/L; −25.5% change from baseline) when compared to the A>C (−0.7 mmol/L) and A/A (−0.4 mmol/L) carriers (25). Further, in a crossover design, only the *CYP7A1* SNP rs3808607-C/C homozygotes showed higher serum TC (4.6 *vs.* 4.0 mmol/L, respectively) and LDL-C (2.2 *vs.* 1.6 mmol/L, respectively) levels after 3 weeks of high-fat (40% en) versus low-fat (22% en) isocaloric diet intakes (56). Also, among 174 normolipidemic subjects, the presence of the C allele, when compared to the A/A genotype, was associated with about 63% higher plasma TC concentrations after an increased intake of dietary cholesterol (742 ± 114 mg/day) based on pooled data from 8 free-living and controlled trials (57). *CYP7A1* SNP rs3808607 was, however, associated with circulating TG but not cholesterol responses to 6-8 weeks of reduced-fat diet intake in a group of 82 dyslipidemic Brazilian males (94).

3.3.14 FABP2 polymorphisms modulate circulating TC and HDL-C responses to low-calorie diet, high saturated fat, and eicosapentaenoic acid intakes

Fatty acid-binding protein 2 (FABP2) is an intracellular protein in the small intestine principally involved in long chain FA uptake and transport processes. Polymorphisms within the *FABP2* gene have previously been linked to fat oxidation, obesity, and insulin resistance, all of which are contributing factors to cardiovascular health (95).

FABP2 SNP rs1799883-C/C (Ala54) homozygotes showed lower serum TC levels, by −6.2% and −7.6% from baseline values, respectively, with the consumption of hypocaloric (~1500 kcal/day) diets low in fat (27% fat, 52% CHO, 20% protein) and

CHO (38% CHO, 36% fat, 26% protein) (58). With a higher (≥53.2 g/day) intake of SFA, carriers of the *FABP2* SNP rs1799883-T (Thr54) allele showed lower circulating HDL-C/TC ratio than did the C/C homozygotes (26). The rs1799883-T allele carriers, however, showed a greater increase in serum HDL-C levels (+30.7% from baseline) relative to the C/C homozygotes (+17.6% from baseline) after 8 weeks of 2 g/day pure eicosapentaenoic acid supplementation intakes (59). The association between *FABP2* SNP rs1799883, as well as SNP rs2282688 and increased circulating HDL-C levels with the intake of medium chain triglycerides was also more recently established in a 2-week dietary intervention (60).

3.3.15 HMGCR polymorphisms modulate circulating cholesterol responses to high polyunsaturated fat, dietary fibre, total fat, and saturated fat intakes

The 3-hydroxy-3-methylglutaryl-coA reductase (HMGCR) is a rate-limiting enzyme in the endogenous synthesis of cholesterol that converts HMGC to mevalonate.96 This enzyme is the target of the cholesterol-lowering drugs statins (97).

In a cross-sectional trial involving 23,011 individuals, only the *HMGCR* SNP rs17238540-T/T homozygotes showed lower serum TC (6.1 *vs.* 6.2 mmol/L, respectively) and LDL-C (3.9 *vs.* 4.0 mmol/L, respectively) levels with higher (>7.3% en) versus lower (<4.7% en) PUFA as well as higher (>22.0 g/day) versus lower (<14.1 g/day) fibre intakes. With higher (>14.3% en) and lower (<10.3% en) SFA intakes, respectively, however, the rs17238540-T/T homozygotes showed higher TC (6.2 and 6.1 mmol/L) and LDL-C (4.0 and 3.9 mmol/L) levels (27). Carriers of the same genotype also showed increased serum HDL-C levels (1.45 *vs.* 1.40; 1.46 *vs.* 1.40; and 1.44 *vs.* 1.40 mmol/L,

respectively) with higher compared to lower total fat; SFA; and fibre intakes, whereas the rs17238540-G allele carriers only showed a weak negative correlation between HDL-C levels and PUFA intake (27). These data accordingly suggest that carriers of the *HMGCR* SNP rs17238540-T/T genotype may respond better to, and benefit more from, dietary portfolios of PUFA and fibre intakes.

3.3.16 LDLR polymorphisms modulate circulating HDL-C and TC responses to high carbohydrate/ low fat diet intake

Low-density lipoprotein receptor is a key cell surface protein, the main ligand of which is LDL, functions as a mediator for the transport of cholesterol-rich lipoprotein particles into cells (98).

Male and female homozygotes for the *LDLR* SNP rs285-T allele (most publication call this *Pvu II*-P1P1 genotype, where P1 and P2 alleles respectively indicate absence and presence of *Pvu II* restriction site) showed higher serum HDL-C levels (1.5 mmol/L) on a 70% en CHO and 15% fat diet relative to a 54% CHO and 31% fat washout diet (1.4 mmol/L) (54). Whereas only female carriers of the same genotype also showed lower TC levels after the HC/LF compared to washout diet (3.4 *vs.* 4.1 mmol/L, respectively), only male carriers of the rs285-C allele (P2) showed lower TC levels compared to the rs285-T/T homozygotes (3.3 *vs.* 3.9 mmol/L, respectively) during the washout diet, indicating a gender specific modulations (54).

3.3.17 LIPC polymorphisms modulate circulating HDL-C responses to high and low total fat, and fatty acid, intakes

E*ncoded by the* LIPC *gene,* hepatic lipase *is* a lipolytic enzyme synthesized and secreted by hepatocytes, and involved in the reverse cholesterol transport, as well as hydrolysis of TGs and phospholipids of all major lipoprotein particles (99).

Male Spanish children carrying the *LIPC* SNP rs1800588 (-514C>T) minor allele T showed higher plasma HDL-C levels (average ~1.6 mmol/L) with the highest consumption (medium and upper tertiles) of total fat (44-64% en), SFA (16-27% en), MUFA (18-29% en), and PUFA (7.4-17% en) compared to the rs1800588-C/C male homozygotes (average ~1.5 mmol/L) (29). The rs1800588-C homozygosity in African American females was also associated with ~0.1 mmol/L lower plasma HDL-C levels versus the C>T and T/T genotypes with low intakes of total fat (28). Although, with high fat intake, *LIPC* SNP rs1800588-C>T heterozygosity was associated with lower HDL-C levels (by ~0.1 mmol/L). Moreover, the rs1800588-T homozygosity in African American males was associated with ~0.1 mmol/L higher HDL-C levels than other genotypes with high fat intake. Previously, Tai et al. (30) reported higher plasma HDL-C levels (1.3 *vs.* 1.1 mmol/L, respectively) in Indian homozygotes for the *LIPC* rs1800588-T allele with <30% en, but ~20% lower HDL-C levels with ≥30% en, dietary fat intake in comparison to the C/C homozygotes. In a more recent crossover study, however, Tucker et al. (50) was not successful at showing significant interactions between *LIPC* SNPs rs1800588 or rs2070895 and intakes of commercially available whole-grain wheat or white bread products.

3.3.18 LPL polymorphisms modulate circulating cholesterol responses to high total fat, fatty acid, and high carbohydrate/low fat diet intakes

LPL is a lipolytic enzyme attached to endothelial cells in capillaries. Upon binding to apolipoprotein CII on chylomicrons and very low-density lipoproteins, LPL hydrolyzes TGs into free FAs and monoglycerides, and promote their cellular uptakes (100). Polymorphisms within the *LPL* gene have been repeatedly associated with differences in circulating lipid profile and CVD risk (101).

Caucasian, but not African American, carriers of the *LPL* SNP rs328-C allele (in many articles referred to as 447Ser or 447S) showed higher plasma HDL-C levels (1.4 mmol/L) with high total fat, SFA, and MUFA (g/day) intakes, whereas the rs328-G/G homozygotes (in many articles referred to as 447Stop or 447X allele) showed lower HDL-C levels (1.3 mmol/L) with higher intakes of these fats among 11,645 individuals from the Atherosclerosis Risk in Communities (ARIC) cohort (28). This *LPL* gene-diet interaction was confirmed in a 6-day dietary intervention among healthy Chinese individuals, where the *LPL* SNPs rs328 and rs320 (in many articles referred to as Hind III) were tested for changes in serum lipid levels in response to an HC/LF (70% CHO, 15% fat, 15% protein) diet (61). There, the rs328 major allele C female homozygotes (presented in the article as S447S), but not the G allele (in the article as 447X) carriers, showed lower serum TC (3.4 *vs.* 3.6 mmol/L, respectively) and LDL-C (1.5 *vs.* 1.8 mmol/L, respectively) levels after versus before the HC/LF intake. Also, only the rs320 major allele T female homozygotes (in the article as H+/H+) showed lower TC (3.4 *vs.* 3.2 mmol/L, respectively), while male carries of the rs320-G allele (in the article as H-C)

showed higher HDL-C (1.4 *vs.* 1.2 mmol/L, respectively), levels after versus before the HC/LF diet. The association between *LPL* genotypes and HDL-C responses to dietary intakes was more recently confirmed in two large Korean cohorts that involved CHO (31) and FA (32) diets. In contrast, a 2006 crossover study involving 35 adults (84) observed no association between the *LPL* SNP S447X and lipid responses to SFA or PUFA diets.

3.3.19 LXRA polymorphisms modulate circulating TC and LDL-C responses to high dietary cholesterol intakes

Liver X receptor is a transcription factor involved in cholesterol, FA, and glucose metabolism mainly in liver and kidneys. Two major isoforms of liver X receptor, α and β, have been characterized (102).

In French Canadian hyperlipidemic men with high cholesterol (>290 mg/day) intake, higher plasma TC (~6.7 mmol/L) and LDL-C (~4.2 mmol/L) levels were associated with the *LXRA* SNPs rs12221497 (-115G>A) A, rs61896015 (-840C>A) A, and rs3758674 (-1830T>C) C alleles, in comparison to the G/G, C/C, and T/T homozygosity, respectively, all of which genotypes showed lower TC (~6.1 mmol/L) and LDL-C (~3.8 mmol/L) levels (33). This cross-sectional study was the first, and probably only, to determine an association between *LXRA* SNPs and circulating cholesterol responses to a cholesterol-rich diet.

3.3.20 NFKB1 polymorphisms modulate circulating HDL-C responses to high polyunsaturated fat intake

Nuclear factor kappa B (NF-κB) is a transcription factor known for its role in a series of biological processes, mainly as a regulator of inflammation through the expression of *tumor necrosis factor alpha* (TNF-α). NF-κB activation has been frequently shown to be mediated by n-6 and n-3 PUFAs (103).

In a 2009 cross-sectional study involving diabetes-free and diet-treated diabetes individuals, higher plasma HDL-C levels were associated with homozygosity for *NFKB1* SNP rs28362491 (-94Ins/Del ATTG) Ins allele during high PUFA intake, where an increase of 1% en from PUFA leaded to a 0.03 mmol/L increase in HDL-C levels (34). In the homozygotes for rs28362491-Del allele, however, PUFA intake and HDL-C levels were inversely correlated.

3.3.21 NPC1L1 haplotype modulates circulating TC and LDL-C responses to plant sterol intake

Niemann-Pick C1 Like 1 (NPC1L1) protein is the membrane transporter moving free cholesterol into enterocytes, and is the target of the cholesterol absorption-lowering drug ezetimibe (104). In pharmacological studies, polymorphisms in the *NPC1L1* gene have been shown to modulate circulating TC and LDL-C levels, thus affecting CVD risk (105,106).

In hypercholesterolemic men, carriers of the *NPC1L1* haplotype rs2072183-G and rs10264715-A alleles showed 1.3- and 2.4-fold greater reductions in plasma TC and

LDL-C levels, respectively, than the wild type allele carriers in response to a 4-week of 2.0 g/day PS intake (43). These data reveal a role of *NPC1L1* SNPs rs2072183 (872C>G) and rs10264715 (3929G>A) in augmenting the cholesterol lowering, and thus cardio-protective influence, of PS intake in carriers of certain *NPC1L1* genotypes.

3.3.22 PPAR polymorphisms modulate circulating cholesterol responses to high polyunsaturated, saturated, and total fat intakes

Peroxisome proliferator-activated receptors **(**PPARs) are transcription factors, of which isoforms α, δ, and γ are extensively described. PPARs are involved in regulating the gene expression in lipid, glucose, and energy metabolism pathways (107).

Homozygosity for the *PPARA* SNPs rs6008259-A allele was associated with lower plasma TC (5.4 mmol/L) and LDL-C (3.4 mmol/L) levels relative to the G allele (5.5 and 3.6 mmol/L, respectively) in diets high in n-6 PUFA (>7.99 g/day) in Caucasians (35). In African American participants of the same study, rs3892755-T allele homozygosity was associated with lower TC (5.4 mmol/L) and LDL-C (3.4 mmol/L) levels with >0.32 g/day n-3 PUFA intake versus C allele (5.6 and 3.5 mmol/L, respectively). Among 632 French Canadian men, however, *PPARA* SNP rs1800206 was not associated with influences on cholesterol levels in interaction with dietary fat intake (108), or a 4-week intake of Step I diet plus low (0.3) and high (1.0) PUFA:SFA ratio (109).

In a cross-sectional study involving 340 healthy French Canadians, the *PPARD* SNP rs2016520 (-87T>C) C allele was associated with higher plasma HDL-C levels (1.2 *vs.* 1.0 mmol/L, respectively) and reduced TC/HDL-C ratio (8.0 *vs.* 10.0, respectively) in

individuals consuming less than 34.4% en from fat, in comparison with the T/T homozygotes (36). According to this observation, carriers of the SNP rs2016520-C allele may be at lower risk of developing CVD, especially when consuming a low-fat diet.

In a 2011 parallel design study, *PPARG* SNP rs1801282-G allele (also known as Ala when the SNP is represented as Pro12Ala) was associated with higher plasma TC (6.1 *vs.* 5.4 mmol/L, respectively) and LDL-C (4.1 *vs.* 3.3 mmol/L, respectively) levels in comparison to the C/C (Pro/Pro) genotype on a low dietary PUFA:SFA ratio (≤0.33) during a 4-week SFA-rich diet intake (62). Previously, among 656 women from the Nurses' Health Study, homozygotes for the *PPARG* SNP rs1801282-C (Pro) allele showed lower plasma TC levels (5.5 *vs.* 5.9 mmol/L) with higher relative to lower total fat intake (40.4% *vs.* 28.3% en, respectively). On the same highest versus lowest quintiles of fat intake, the Pro/Pro homozygotes showed lower plasma HDL-C levels (1.5 *vs.* 1.6 mmol/L, respectively), whereas carriers of the rs1801282-G (Ala) allele showed the opposite trend (1.7 *vs.* 1.5 mmol/L) (37). More recently, *PPARG2* SNP rs10856710-C allele Inuit carriers showed higher plasma TC and LDL-C levels with higher intakes of total fat (β coefficients = 0.0069 and 0.0068 for C/C homozygotes in TC and LDL-C, respectively) and SFA (β coefficients = 0.0195 and 0.0186 for C/C homozygotes in TC and LDL-C, respectively) (21). No gene-diet interactions were, however, reported for certain *PPARG* SNPs with a 14-week calorie restriction (~1200 kcal/day) in 95 Japanese women (110) or different PUFA/SFA ratio of intake among 2,120 Singaporean individuals of different ethnic backgrounds (111).

3.3.23 TNFA polymorphisms modulate circulating cholesterol responses to high polyunsaturated and saturated fat intakes

TNF-α is a multifunctional macrophage-secreted inflammatory cytokine, the higher circulating levels of which have been associated with insulin resistance, cancer, and CVD (112,113).

In a cross-sectional study involving 109 diabetic individuals (38), carriers of the *TNFA* SNP rs361525 (-238G>A) A allele showed higher serum HDL-C levels with increased PUFA intake (β coefficient = 0.06 mmol/L per 1% en from PUFAs), whereas SNP rs361525-G/G homozygotes and SNP rs1800629 (-308G>A) A allele carriers showed the opposite trend (β coefficients = −0.03 and −0.07, respectively). Similarly, in a more recent cross-sectional study involving over 200 normal-weight and obese black South African women (39), *TNFA* SNP rs1800629-A allele was associated with a lower serum TC/HDL-C ratio (from ~4.0 to 2.0) and higher LDL-C levels (from ~1.5 to 3.5 mmol/L) with increased n-3 ALA (0.0-0.5% en) and PUFA (0.0-20% en) intakes, respectively. Among white South African females with increased dietary SFA intake (4-16% en), *TNFA* SNP rs1800629-G/G homozygotes showed lower (from ~5.2 to 4.7 mmol/L), whereas the A allele carriers showed higher (from ~4.3 to 5.0 mmol/L), serum TC levels (40). Moreover, *TNFA* SNP rs361525-G>A black and white South African female carriers showed higher serum TC/HDL-C ratio (from ~2.4 to 3.5), and lower HDL-C levels (from ~2.0 to 1.2 mmol/L), with increased n-6:n-3 PUFA ratio (0-100) intakes, whereas G/G homozygotes showed the opposite trend (41). Together, these data suggest that circulating cholesterol responses to PUFA intake may differ as a result of a pre-

existing *TNFA* genotypes, although the role of TNF in cholesterol metabolism is yet to be clearly established.

3.3.24 UCP1 and UCP3 polymorphisms modulate HDL-C and TC responses to low-calorie and low carbohydrate diet intakes

Uncoupling proteins (UCPs) are mitochondrial inner membrane transporters primarily involved in energy metabolism through participating in the electron transport chain, diet-induced thermogenesis, and FA oxidation. UCPs have hence been of an importance in body weight and lipid homeostasis (114).

The *UCP1* SNP rs1800592 (-3826A>G) G allele was, in obese women, associated with a smaller reduction in serum HDL-C levels (−0.06 mmol/L) in comparison with the A/A homozygotes (−0.26 mmol/L) versus baseline in responses to an 8-week LCD (1222 kcal/day) intake (63). In the same study, however, LCD was successful in reducing TC and LDL-C levels to the same degree in individuals of all genotypes. In another caloric restriction study (~1500 kcal/day) (64), homozygosity for the *UCP3* SNP rs1800849 (-55C>T) C allele was associated with lower serum TC levels versus baseline (4.7 *vs.* 5.1 mmol/L, respectively) when obese individuals consumed a low CHO diet (38% CHO, 36% fat, 26% protein) but not a low fat diet (53% CHO, 27% fat, 20% protein).

3.4 GENERAL REMARKS AND FUTURE DIRECTIONS

Compared to the literature on GWASs, which associate genetic variants with the intrinsic levels of circulating cholesterol, fewer studies test genetic variantions in relation to differing cholesterol responses to dietary intakes. Further, none of these studies utilized a

GWAS approach, and the emerging information on the gene-diet interactions is derived from candidate gene studies in "classical" epidemiologic settings (cross-sectional, case-control, and cohort) or from dietary interventions (parallel and crossover) of moderate sizes. Variants in 28 genes have been associated with responses of circulating cholesterol levels to various diets. Sixteen of the genes associated to modulate gene-diet interactions are also reported to be associated with impacts on intrinsic circulating cholesterol levels in GWAS, including *ABCA1*, *ABCG5*, *ABCG8*, *APOA1*, *APOA4*, *APOA5*, *APOC3*, *APOE*, *CD36*, *CETP*, *CYP7A1*, *HMGCR*, *LDLR*, *LIPC*, *LPL*, and *NPC1L1*. This shows that genetic variations in the cholesterol metabolism pathways can influence intrinsic levels as well as an individual's response to the diet.

Notably, most of the gene-diet interactions described are isolated observations and replication is warranted. The lack of replication is likely due to the wide spectrum in designs of existing studies, which vary in durations, diets, candidate genes/genotypes, participants' numbers, locations, and ethnic backgrounds, as well as dietary assessment methodologies. For example, the number of participants ranged between $n = 28$ in a randomised controlled trial (50) to $n = 23{,}011$ individuals in a cohort study (27). Moreover, while one study was carried out as a full-feeding design for 13 days (54), another was established on dietary changes over 8 years (22). We anticipate that the next wave of studies will improve on design and statistical power, for instance by utilizing GWAS or genome sequencing methodologies in large observational studies. Moreover, larger sample sizes should be anticipated for well-controlled dietary interventions.

The lowering of circulating cholesterol levels through dietary interventions is relevant to prevention of CVD regardless of a person's genotype (2). However, the reviewed studies indicate that carriers of certain genotypes within cholesterol-related genes respond far better to a given dietary intervention than others; the clinical impacts of these further improvements seem to be significant for most cases reported. For example, a 3.9-fold greater reduction in serum LDL-C levels was observed in hypercholesterolemic men carrying SNP rs4148217-A, but not the other allele in the *ABCG8* gene with the intake of 2.0 g/day PS for 4 weeks (43). Generally, beneficial effects were in excess of 15% compared to individuals with genotypes associated with lower responses to any diets (**Figure 3.1**). These effect sizes are distinct from the ones reported to determine intrinsic cholesterol levels in GWASs, which are generally estimated at 1-2% of the observed phenotype and are therefore considered too small to be clinically meaningful (2). This indicates that variants determining gene-diet interactions might have a higher clinical relevance compared to the ones influencing the intrinsic levels (**Figure 3.1**).

Moreover, existing studies do not report on gene-gene-diet interactions, where epistatic effects can potentiate the impact of any given common variant of lower influence. Epistatic effects have been shown to determine the impact size for the *APOA5* SNP rs75423577C>T in hyperlipoproteinaemia type 5, where a rare combination of common SNPs elevates the risk to a level normally seen in monogenic events (**Figure 3.1**) (2). With improved study designs we anticipate that epistatic relations will be discovered and could emerge as some of the most powerful diagnostic tools in preventative personalized medicine.

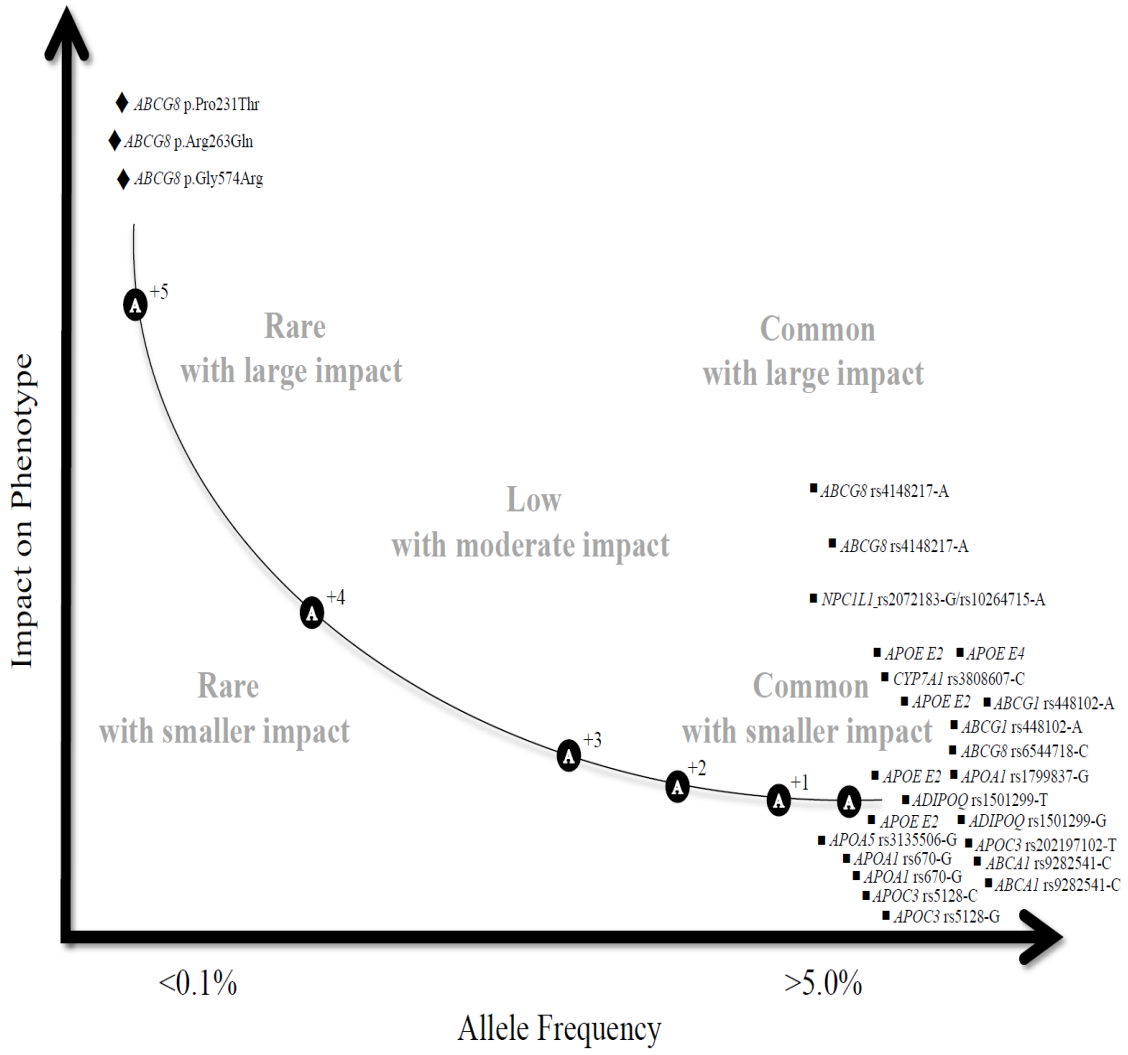

Figure 3.1 Effect sizes of circulating cholesterol responses to dietary intakes in association with genetic variations. Black squares (■) indicate clinically significant effect sizes. The highest effect size is reported as a 3.9-fold greater reduction in serum LDL-C levels observed in hypercholesterolemic men carrying SNP rs4148217-A allele in the *ABCG8* gene. Most gene-diet interactions reported today are associated with common variants. Through epistatic interactions of multiple common variants effect sizes could dramatically increase, while the frequency for each combination decreases (2). This is illustrated for one hypothetical variant indicated by the black circles (●), which we modeled after the epistatic effects described for the *APOA5* SNP rs75423577-C>T (2). The impact of the hypothetical variant A increases when it is present with different combinations of other variants of low impact sizes and can reach levels only known from monogenic events. As examples of rare variants with high impact sizes on the phenotype of sitosterolemia, three variants in the *ABCG8* gene are indicated as black rectangles (♦) (68). However, whether or not they determine gene-diet interactions remains to be closely investigated.

Although we predict that the impact of some genetic discoveries will eventually translate into preventative and clinical health practices, available knowledge is modest and more evidence is needed to validate the current as well as future findings. This can only be achieved through a fusion of multidisciplinary data, where epidemiological observations create a hypothesis to be validated though biological studies and, ultimately, human dietary interventions. Only gene-diet interaction validated through well-controlled nutritional trials should be used to instruct genetic-targeted diet modifications in future public health practices.

3.5 CONCLUSION

In the postgenomic era, a promising body of evidence emerged to highlight a clinically relevant role of the genomic architecture in modulating circulating levels of cholesterol, a classic biomarker of CVD, in response to dietary intakes. This gene-diet interaction is a powerful step toward deepening understanding of the diet-health framework. The existing knowledge, however, is modest, and in order to incorporate genetically targeted dietary modifications in future public health practices, early observations must be replicated and validated in biological and clinical studies.

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MMHA conducted the literature search and wrote the manuscript. All authors contributed to revisions of the manuscript and approved the final version. The authors declare no conflict of interest in association with the content of this work.

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BRIDGE TO CHAPTER IV

Milk and dairy products are recommended for consumption "as a group of low-fat products" in the level of 2-4 servings/day by most food guides, however, in reality a combination of low-fat and regular dairy from the marketplace is usually consumed by the general public. The majority of controlled dietary intervention studies, furthermore, seem to assess individual rather than a combination of dairy product intakes, and to overlook the influence of dairy on fatty acid (FA) profile in blood that can also act as a dietary biomarker to verify compliance particularly in free-living designs. Data in Chapter IV take these points in consideration as they substantiate the lipid- and FA-modulating impacts of the Canadian recommended level of dairy intake in the entire population of this research. The following manuscript has been published in the British Journal of Nutrition 2015;113:435-44.

CHAPTER IV

MANUSCRIPT 2

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RECOMMENDED DAIRY PRODUCT INTAKE MODULATES CIRCULATING FATTY ACID PROFILE IN HEALTHY ADULTS: A MULTI-CENTRE CROSSOVER STUDY

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4.1 ABSTRACT

Dairy products are rich sources of an array of fatty acids (FAs) that have been shown individually and in certain clusters to exert varying effects on cardiovascular health, for which circulating lipid profile is a powerful biomarker. Whether the profile of these FAs is reflected in blood upon short-terms of intake, possibly contributing to lipid-related health impacts of dairy, remains to be fully established. Our objectives were to assess a recommended dairy consumption in relation to circulating FA and lipid profiles, including evaluation of certain FAs in dairy fat as potential biomarkers of intake. In a free-living, multicentre, crossover design, 124 healthy individuals consumed 3 servings/day of commercial dairy (1% fat milk, 1.5% fat yogurt, and 34% fat cheese) or energy-equivalent control (fruit and vegetable juice, cashews, and a cookie) products for 4 weeks each, separated by a 4-week washout period. Plasma FA and serum lipid profiles were assessed by standard methods at the end of each dietary phase. After 4 weeks of intake, plasma levels of FAs pentadecanoic acid (C15:0) and heptadecanoic acid (C17:0) were higher (0.26 *vs.* 0.22% and 0.42 *vs.* 0.39% of total identified FAs, respectively) after DAIRY than after CONTROL ($P < 0.0001$). This was accompanied by small but significantly higher serum LDL-C levels after dairy vs. control (+0.08 mmol/L, $P = 0.04$). In conclusion, intake of 3 servings/day of conventional dairy products may modify certain circulating FA and lipid profiles within 4 weeks, where C15:0 and C17:0 may be potential short-term biomarkers of intake.

4.2 INTRODUCTION

Consumption of dairy products is advocated by most dietary guidelines worldwide, as part of a balanced nutritional pattern and healthy overall lifestyle, for optimal health status (1, 2). Still, epidemiological and dietary intervention studies reveal a somewhat equivocal picture of evidence regarding the impact of dairy intake on major public health concerns, including cardiovascular disease (CVD) (3-10), for which circulating lipid profile is a classic biomarker. The majority of the studies in the current literature, however, seem to overlook the lipid- and thus cardiovascular health-modulating influence of a key component in dairy foods, fatty acids (FAs).

Milk and other dairy products are naturally rich in a multitude of dietary FAs (11) that have independently been shown to exert varying health effects (12). Intake of medium chain FAs (MCFAs), of six to twelve carbon atoms (C6:0-12:0), that typically constitute 8-22% of total FAs in dairy fat may lead to greater energy expenditure and, thus, better weight management (13, 14). Palmitic acid (C16:0), the predominant saturated FA (SFA) in dairy, representing 22-35% of its fat content, elevates circulating low-density lipoprotein cholesterol (LDL-C) levels, with unclear effects however on the risk of CVD (15). Oleic acid (C18:1n-9), the main monounsaturated FA (MUFA) in most foods, and which usually represents 20-30% of dairy FAs, may protect against CVD via multiple mechanisms of action (16, 17). Whether or not the cluster of these FAs is reflected in blood upon dairy intake, possibly contributing to its impacts on lipid levels, is yet to be fully established.

On the other hand, some FAs specific to dairy foods have been used as biomarkers of long-term dairy fat intake in observational studies (8, 18-21) and on some occasions been associated with altered health states (15, 22-25). Of these FAs, pentadecanoic acid (C15:0) and heptadecanoic acid (C17:0) are traditional examples (18), whereas *trans*-palmitoleic acid (*trans*-16:1n-7) has only recently emerged as a potential biomarker of dairy intake (26).

The objective of this study was to examine the impact of the recommended 3 servings/day intake of commercially-available dairy products, over 4 weeks, on circulating FA and lipid profiles in healthy individuals. The study further sought to evaluate the capacity of certain odd-chained FAs specific to dairy fat to serve as potential short-term biomarkers of intake.

4.3 EXPERIMENTAL METHODS

4.3.1 Study population

A multicentre study involving two Canadian research institutes, the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN) in Winnipeg, Manitoba, and the Institute of Nutrition and Functional Foods (INAF) in Quebec City, Quebec, was conducted to investigate the impact of short-term dairy products consumption on a constellation of risk markers of coronary heart disease (CHD) in healthy population. The experimental methods have been described elsewhere (27). One hundred and thirty seven apparently healthy individuals were recruited in January through September 2011 by the use of advertisements, e-newsletters, and word of mouth. Eligibility criteria included the

age of 18-70 years and two consecutive measures of 12-h fast circulating high-sensitivity C-reactive protein (*hs*-CRP) levels, an established biomarker of inflammation and hence cardiovascular health (28), and the primary study outcome, between 1.0 and 10.0 mg/L taken over 2 weeks. An *a priori* defined lower limit of 2.0 mg/L for *hs*-CRP was changed to 1.0 mg/L during the study to facilitate recruitment. Exclusion criteria included smoking, fluctuations in body weight, documented CVD, diabetes, monogenic dyslipidemia, or any endocrine or gastrointestinal disorder, routine use of lipid-lowering, anti-hypertensive, or anti-inflammatory medications, anemia, and allergies or restrictions to dairy or other food products provided in the study. Individuals who took dietary supplements, in particular vitamin D, calcium, fish oil, omega-3, probiotics, or calcium-enriched or soy products were asked to stop the intake prior to starting the study. Female volunteers did not take part in the study when they were pregnant or breast-feeding. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Bannatyne Campus Research Ethics Boards (University of Manitoba) and by the CERUL (Research Ethics Board) of Laval University. Written informed consent was obtained from all subjects/patients. The study was registered on Clinicaltrials.gov under identifier #NCT01444326.

4.3.2 Study design and diets

A randomized, free-living, crossover clinical trial was conducted concurrently at the RCFFN and the INAF. The study consisted of a 2-week run-in period followed by either of two diet sequences, DAIRY-CONTROL or CONTROL-DAIRY, where each treatment

phase (DAIRY and CONTROL) lasted for 4 weeks and was separated by a 4-8 week washout period. Participants were randomized to the diet sequences by a computer-generated number order based on their screening circulating *h*s-CRP levels (with 1.0-2.0 and 2.0-10.0 mg/L levels considered as low and high, respectively), gender, and centre to ensure adequate balance of the subgroups within each sequence. Prior to the study start, and when required throughout, participants received, and were asked to precisely comply with, general dietary advice in accordance with the nutritional guidelines for healthy eating, including lower intakes of SFA (<10% en), *trans* fat (<1% en), dietary cholesterol (<200 mg/day), and sodium (<2300 mg/day). With recommendations for 25-35% of daily energy, total fat intake was not restricted, however, substituting saturated fats for unsaturated fats, as well as restricting consumption of processed and energy-dense foods, were advocated. Participants were also instructed to limit their alcoholic beverages to 1 drink/day, or a maximum of 5 drinks/week, and caffeinated beverages to 2 cups/day, as well as to refrain from consuming soy products and dairy in hidden sources (e.g. pizza, pastry, pancake, or cream). The recommendations highlighted that this study was not a weight loss program and that participants should keep their level of physical activity constant at all times. A specific recommendation was set to limit the intake of dairy products to a maximum of 2 servings/day during the run-in and washout periods. During the study's DAIRY phase, participants were provided with conventional dairy products, including low-fat (1%) milk, low-fat (1.5%) creamy stirred yogurt (fruit flavours), and regular (34%) cheddar cheese on a weekly basis, and were asked to incorporate 3 servings of these products into their everyday diet as follows: 375 ml/day of milk, 175 g/day of yogurt, and 30 g/day of cheese. During the CONTROL phase, participants were

provided with energy-matched control products each week, and were asked to consume 290 ml of fruit juice, 156 ml of 100% vegetable juice, 20 g of cashews, and one 39 g cookie (prepared in our metabolic kitchens) everyday. To reach a net difference of 2-3 servings per day of dairy, as recommended for adults by Canada's Food Guide (1), no intake of dairy foods was allowed during the CONTROL phase. **Table 4.1** summarizes the composition of the study's food products.

4.3.3 Compliance to dietary intake

Compliance to the study's dietary protocols was assessed through the use of a validated web-based food frequency questionnaire (FFQ) (29) completed at screening time and the end of each treatment phase. Participants were also provided with checklists on each of their weekly visits and asked to report their daily consumption of the study products, as well as to indicate intakes of alcohol, caffeinated beverages, and medications. Dietary compliance was confirmed by the measurement of serum levels of 25-hydroxyvitamin D [25(OH)D], a marker of vitamin D intake (30), at the end of each dietary phase.

4.3.4 Anthropometry and blood pressure

At screening, as well as the start and end of each dietary phase, body weight and waist circumference were measured according to standardized procedures (31). Resting systolic and diastolic blood pressure measurements were performed after a 10-min rest in a sitting position by an automated blood pressure monitor (BPM 300-BpTRU model; Vital Signs Monitor, VSM MedTech Ltd., Coquitlam, BC, Canada). Blood pressure data are presented as averages of 3 readings.

Table 4.1 Combined nutritional composition of the dairy and control products provided during the intervention[1]

	CONTROL[2]	**DAIRY**[3]
Energy [kJ (kcal)]	1833 (438)	1807 (432)
Total fat (g)	17.1	16.3
SFA[4] (g)	7.6	8.7
MUFA[5] (g)	6.6	4.1
PUFA[6] (g)	1.9	0.4
TFA (g)	0.01	0.08
Cholesterol (mg)	10.2	61.8
Protein (g)	7.7	26.0
Carbohydrate (g)	63.3	45.0
Fibre (g)	3.2	0.0
Sodium (mg)	459.0	465.0
Calcium (mg)	84.0	913.0
Vitamin D (μg)	0.0	5.2

[1] Data in this table, except for cholesterol, are reproduced from ME Labonté *et al.* (27). [2] CONTROL, energy-equivalent control products; includes fruit juice (290 ml), vegetable juice (156 ml), cashews (20 g), and one cookie (39 g). [3] DAIRY, commercial dairy products; includes 1% milk (375 ml), 1.5% yogurt (175 g), and 34% cheese (30 g). [4] Sum of FAs C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, C20:0, and C22:0. [5] Sum of FAs C16:1n-7, C18:1n-9, C20:1n-9, and C22:1n-9. [6] Sum of FAs C18:3n-3, C18:4n-3, C20:5n-3, C22:5n-3, C22:6n-3, C18:2n-6, and C20:4n-6. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; TFA, *trans* fatty acid.

4.3.5 Blood sampling and biochemical parameters

At screening, serum lipid profile was assessed as previously described (32) and *hs*-CRP levels measured by nephelometry (33). At start and end of each dietary phase, two 12-hr fasted blood samples were collected and centrifuged within 1-2 h at 3000 rpm and 4°C for 20 min. Aliquots of serum, plasma, erythrocyte, and leukocyte fractions were immediately prepared and stored at −80°C until analysis. Serum lipid profile, including total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) levels, were determined in duplicates and levels averaged at the end of each dietary phase by a Roche/Hitachi Modular analyzer (Roche Diagnostics, Laval, QC, Canada) using Roche Diagnostics reagents. LDL-C levels were calculated according to the Friedewald formula (34). Framingham 10-year CHD risk scores (FRSs) were estimated by FRS online calculator (35) based on age, sex, systolic blood pressure, TC, and HDL-C values of each participant at the end of each dietary phase.

4.3.6 Fatty acid profile

Plasma total lipids were extracted by the method of Folch (36) using 2:1 v/v chloroform-methanol including 0.01% butylated hydroxytoluene (BHT), and heptadecenoic acid (C17:1) as an internal standard (Sigma-Aldrich, Oakville, ON, Canada). Following extraction, FAs were methylated with methanolic HCL. Separation of FA methyl esters was then completed on a Varian WCOT Fused Silica CP-SELECT FAME column (100 m X 0.25 mm diameter and 0.25 μm film thickness; Varian Canada Inc., Mississauga, ON, Canada) by a Varian 450 gas chromatograph (GC) with a flame ionization detector (FID). The column was operated at 130°C for 2 min, after which the temperature was

raised to 175°C at 25°C/min, held for 25 min, raised again to 240°C at 3°C/min, and held for 10 min. The total run time was 60.47 min, and samples were run with a 20:1 split ratio and a column flow rate of 0.8 ml/min with hydrogen as the carrier gas. Injector and detector temperatures were set at 270°C and 290°C, respectively. Individual FAs were identified via comparisons with known standards (NuChek Prep Inc., Elysian, MN, USA), and were quantified and presented as percentages of total FAs of interest according to the peak area relative to the total area.

4.3.7 Statistical analyses

Statistical analysis was performed by SAS package version 9.2 (SAS Institute Inc., Cary, NC, USA). All values are expressed as means ± SE unless otherwise indicated, with differences considered statistically significant at $P < 0.05$. Impacts of the two dietary treatments (endpoint values) on the outcomes of interest were compared using PROC MIXED procedure for repeated measures, with diet, sequence, and gender as fixed factors, and study centre and subjects as random factors in the selected model, unless otherwise stated. Spearman correlation coefficient analyses were used for assessment of the relation between plasma 25(OH)D and serum lipid levels, as well as between intakes of total SFAs, MUFAs, PUFAs, and MCFAs, as estimated by the FFQs, and their corresponding FA levels in plasma. Abnormally distributed variables were natural log-transformed before statistical analysis.

4.4 RESULTS

4.4.1 Participant characteristics and compliance

Out of the 137 participants who were originally randomized to the study groups, 124 (84 females and 40 males) completed the intervention (**Figure 4.1**). Baseline, represented by the screening time point, characteristics of individuals who completed the study are shown in **Table 4.2**. Seven eligible participants never started the study and six dropped out due to inability to commit to the study protocol or for personal reasons. The FFQ data indicated good compliance to DAIRY, evident by an average of 3.5 servings/day net difference between the intake of dairy and control products ($P < 0.0001$) (**Table 4.3**). Akin to the Milk and Alternatives food group, intakes of Vegetables and Fruit, and Meat and Alternatives, but not Grain Products, food groups were also significantly different between the two dietary protocols ($P < 0.0001$ for both) (**Table 4.3**). With regard to the daily energy and nutrient intakes, participants while on DAIRY consumed more calories, SFAs, dietary cholesterol, protein, calcium, and vitamin D ($P < 0.01$ for all), but less total fat, MUFA, PUFA, total carbohydrates, and fibre in comparison to CONTROL ($P < 0.01$ for all) (**Table 4.4**). Compliance to the dairy intake was confirmed by the significantly higher serum levels of 25(OH)D after DAIRY as compared to CONTROL (76.4 ± 4.9 vs. 67.8 ± 4.9 nmol/L, respectively, $P < 0.0001$).

There was no statistically significant difference in anthropometric measurements and blood pressure ($P \geq 0.08$) between diets (**Table 4.5**). There was also no significant difference between DAIRY and CONTROL in plasma *hs*-CRP levels (27).

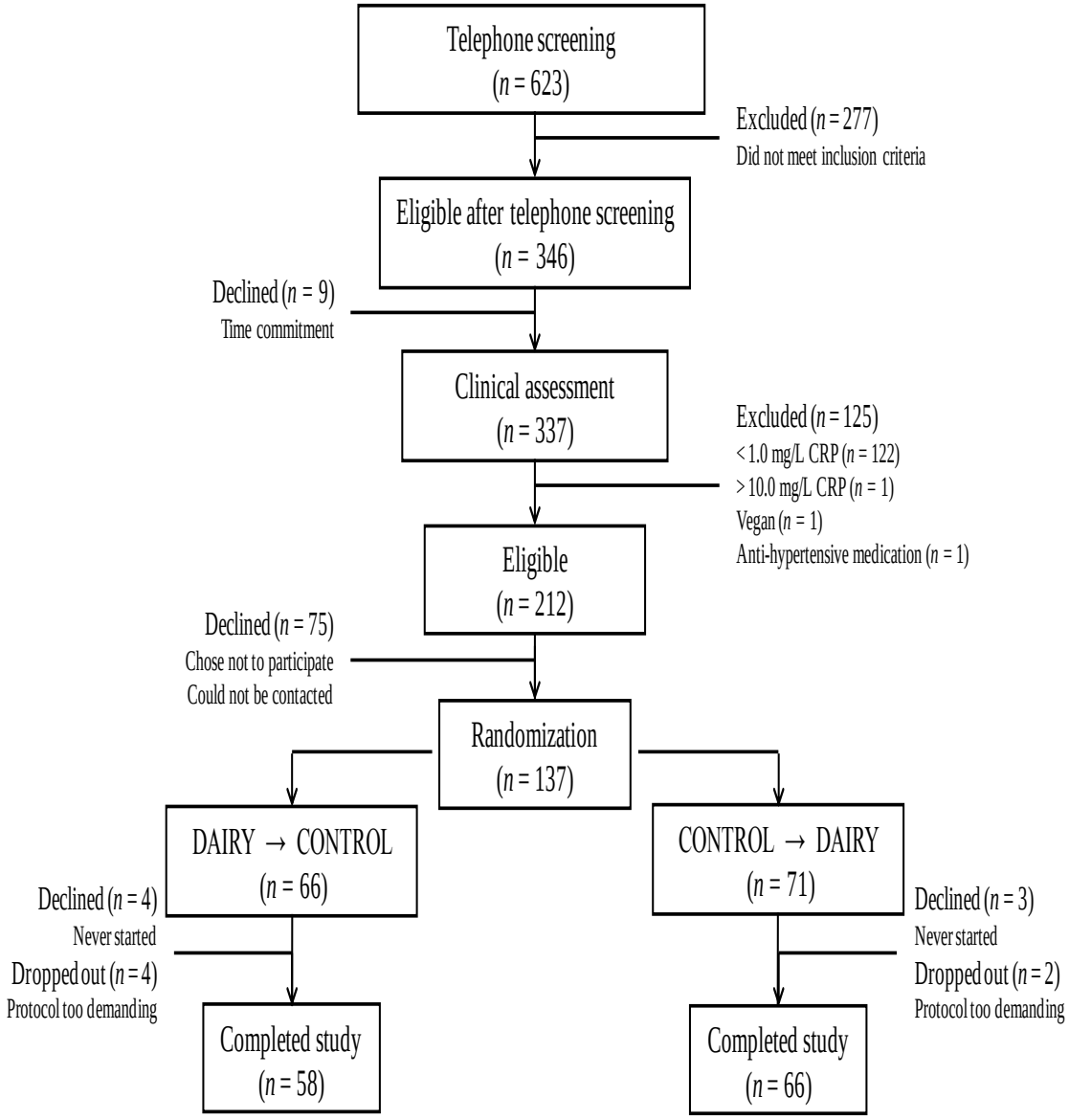

Figure 4.1 Flow chart of the study. CONTROL, energy-equivalent control products; DAIRY, commercial dairy products. CRP, C-reactive protein.

Table 4.2 Baseline characteristics of the study's participants[1]

	Mean	SD	Min-Max
Women [n (%)]	84 (67.7)		
Age (year)	39.3	16.4	18-69
Anthropometric measures			
Body weight (kg)	72.0	15.5	46.3-122.4
BMI (kg/m^2)	25.7	4.2	17.4-36.4
Waist circumference (cm)	87.3	13.5	61.5-118.0
Blood pressure[2] *(mm Hg)*			
Systolic	112.6	13.1	83.7-148.0
Diastolic	66.7	7.9	42.7-84.0
Plasma glucose and lipid levels[2] *(mmol/L)*			
Glucose	5.1	0.4	4.4-6.1
TC	5.2	1.0	3.1-7.9
HDL-C	1.7	0.4	0.9-2.8
LDL-C	2.9	0.9	1.2-5.3
TG	1.4	0.7	0.4-3.2
TC:HDL-C ratio	3.3	1.0	1.8-7.0
LDL-C:HDL-C ratio	1.9	0.9	0.5-4.7

[1] At screening. [2] Values are representatives of a subsample of 60 participants (Québec Centre). BMI, body mass index; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides.

Table 4.3 Daily food group intakes at screening and the end of each dietary phase[1]

Food group	SCREENING	CONTROL	DAIRY	Δ[2]	*P*[3]
		servings/day			
Vegetables and fruit	5.7 (5.1-6.3)	8.3 (7.6-9.0)	5.2 (4.6-5.8)	−3.1	<0.0001
Grain products	4.4 (4.0-4.8)	3.8 (3.4-4.2)	3.8 (3.4-4.3)	0.0	0.67
Milk and alternatives	1.7 (1.5-2.0)	0.0 (0.0-0.0)	3.5 (3.3-3.7)	3.5	<0.0001
Meat and alternatives	2.1 (1.9-2.3)	2.4 (2.2-2.7)	1.9 (1.7-2.1)	−0.5	<0.0001

SCREENING, food and nutrient intakes of the participants prior to the dietary intervention (i.e. at screening time); CONTROL, energy-equivalent control products; DAIRY, commercial dairy products. [1] Values are geometric means with log10 transformation (95% CI). [2] DAIRY − CONTROL. [3] *P* values for DAIRY versus CONTROL. *n* = 119 in SCREENING as dietary data for 5 participants were excluded because of non-plausible energy intakes [<2092 or >14 644 kJ (<500 or >3500 kcal) in women and <3347 or >17 573 kJ (<800 or >4200 kcal) in men]; *n* = 117 in CONTROL as dietary data were missing for 3 participants and excluded for 4 participants because of non-plausible energy intakes (same criteria); *n* = 114 in DAIRY as dietary data were missing for 7 participants and excluded for 3 participants because of non-plausible energy intakes (same criteria).

Table 4.4 Daily energy and nutrient intakes at screening and the end of each dietary phase[1]

Nutrient	SCREENING	CONTROL	DAIRY	*P*[2]
Energy (kJ)	7904.0 (1775.4-2010.1)	7210.0 (1615.8-1837.8)	7758.0 (1738.5-1977.7)	0.003
Alcohol (%)	0.6 (0.4-0.8)	0.4 (0.3-0.6)	0.3 (0.2-0.5)	0.04
Total fat (%)	33.3 (32.3-34.3)	32.4 (31.4-33.5)	30.7 (29.9-31.6)	0.0006
SFA (%)	10.9 (10.5-11.3)	8.5 (8.2-8.8)	10.7 (10.4-11.1)	<0.0001
MUFA (%)	13.3 (12.7-13.8)	14.0 (13.4-14.6)	12.0 (11.6-12.5)	<0.0001
PUFA (%)	6.1 (5.9-6.4)	7.1 (6.9-7.4)	5.3 (5.1-5.6)	<0.0001
TFA (%)	1.2 (1.2-1.3)	1.1 (1.1-1.2)	1.1 (1.0-1.2)	0.29
Dietary cholesterol (mg)	233.3 (216.0-252.0)	168.0 (150.0-188.3)	209.8 (193.8-227.1)	<0.0001
Protein (%)	16.8 (16.3-17.4)	14.1 (13.6-14.7)	18.2 (17.7-18.8)	<0.0001
Total carbohydrate (%)	49.9 (48.6-51.2)	54.5 (53.0-56.1)	51.7 (50.5-53.0)	<0.0001
Fibre (g)	22.1 (20.3-23.9)	22.9 (21.0-25.0)	20.3 (18.3-22.5)	0.001
Sodium (mg)	2629.6 (2457.8-2813.5)	2338.5 (2201.0-2484.6)	2338.1 (2177.2-2510.8)	0.81
Calcium (mg)	1024.3 (933.4-1124.0)	442.1 (410.5-476.1)	1413.8 (1335.1-1497.1)	<0.0001
Vitamin D (μg)	7.5 (6.4-8.6)	3.4 (2.9-4.1)	8.3 (7.5-9.1)	<0.0001

SCREENING, food and nutrient intakes of the participants prior to the dietary intervention (i.e. at screening time); CONTROL, energy-equivalent control products; DAIRY, commercial dairy products. [1] Values are geometric means with log10 transformation (95% CI). [2] *P* values for DAIRY versus CONTROL. *n* = 119 in SCREENING as dietary data for 5 participants were excluded because of non-plausible energy intakes [<2092 or >14 644 kJ (<500 or >3500 kcal) in women and <3347 or >17 573 kJ (<800 or >4200 kcal) in men]; *n* = 117 in CONTROL as dietary data were missing for 3 participants and excluded for 4 participants because of non-plausible energy intakes (same criteria); *n* = 114 in DAIRY as dietary data were missing for 7 participants and excluded for 3 participants because of non-plausible energy intakes (same criteria). SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; TFA, *trans* fatty acid.

Table 4.5 Anthropometry, blood pressure, and biochemical parameters at the end of each dietary phase[1]

	CONTROL		DAIRY		Δ	*P*
	Mean	**SE**	**Mean**	**SE**		
Anthropometric measures						
Body weight (kg)	71.9	1.4	72.2	1.4	0.3	0.08
BMI (kg/m^2)	25.6	0.4	25.7	0.4	0.1	0.08
Waist circumference (cm)	87.2	1.2	87.8	1.3	0.4	0.38
Blood pressure (mm Hg)						
Systolic	109.9	1.3	109.0	1.2	−0.9	0.27
Diastolic	68.9	0.9	69.2	0.9	0.3	0.54
Serum lipid levels (mmol/L)						
TC	5.0	0.1	5.1	0.1	0.1	0.07
HDL-C	1.5	0.0	1.5	0.0	0.0	0.92
LDL-C	2.9	0.1	3.0	0.1	0.1	0.04
TG	1.2	0.1	1.2	0.1	0.0	0.69
TC:HDL-C ratio	3.5	0.1	3.5	0.1	0.1	0.06
LDL-C:HDL-C ratio	2.1	0.1	2.1	0.1	0.1	0.03

CONTROL, energy-equivalent control products; DAIRY, commercial dairy products.
[1] Values are means ± SE. BMI, body mass index; TC, total cholesterol, HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides.

4.4.2 Lipid profile and FRS

Except for LDL-C levels (2.99 ± 0.08 vs. 2.91 ± 0.08 mmol/L, $P = 0.04$) and LDL-C:HDL-C ratio (2.12 ± 0.08 vs. 2.07 ± 0.08, $P = 0.03$), which were slightly higher after DAIRY relative to CONTROL, no difference was observed for the serum lipid levels ($P \geq 0.06$) (**Table 4.5**). Correlation coefficient analyses detected an inverse relationship between plasma 25(OH)D levels and TC:HDL-C ($r = -0.24$, $P = 0.007$) and LDL-C:HDL-C ($r = -0.22$, $P = 0.01$) ratios after DAIRY (**Figure 4.2**). No significant differences in FRS values were observed with DAIRY in comparison to CONTROL (5.38 ± 0.49 vs. 5.54 ± 0.51 %, respectively, $P = 0.32$)

4.4.3 Plasma fatty acids

At the start of the trial, no statistically significant differences were observed in plasma FA levels, except for *trans*-16:1n-7 levels, which were slightly higher before DAIRY than before CONTROL (0.25 ± 0.00 *vs.* 0.24 ± 0.01 % of total identified FAs, $P = 0.003$, not shown). **Table 4.6** shows circulating FA levels at the end of each dietary phase. Higher plasma levels of FAs C15:0 and C17:0 (0.26 ± 0.01 *vs.* 0.22 ± 0.01 % and 0.42 ± 0.01 *vs.* 0.39 ± 0.01 % of total identified FAs, respectively, $P < 0.0001$ for both), and a trend of higher *cis*-9,*trans*-11-18:2n-6 levels (0.18 ± 0.01 *vs.* 0.16 ± 0.01 % of total identified FAs, $P = 0.06$) were observed with DAIRY versus CONTROL. There was no significant baseline CRP x diet interaction in determining the change in C15:0 ($P = 0.88$ for CRP x dairy), C17:0 ($P = 0.72$), or CLA ($P = 0.87$). In other words, these FAs were higher (or showed a trend of higher levels in case of CLA) after DAIRY (*vs.*

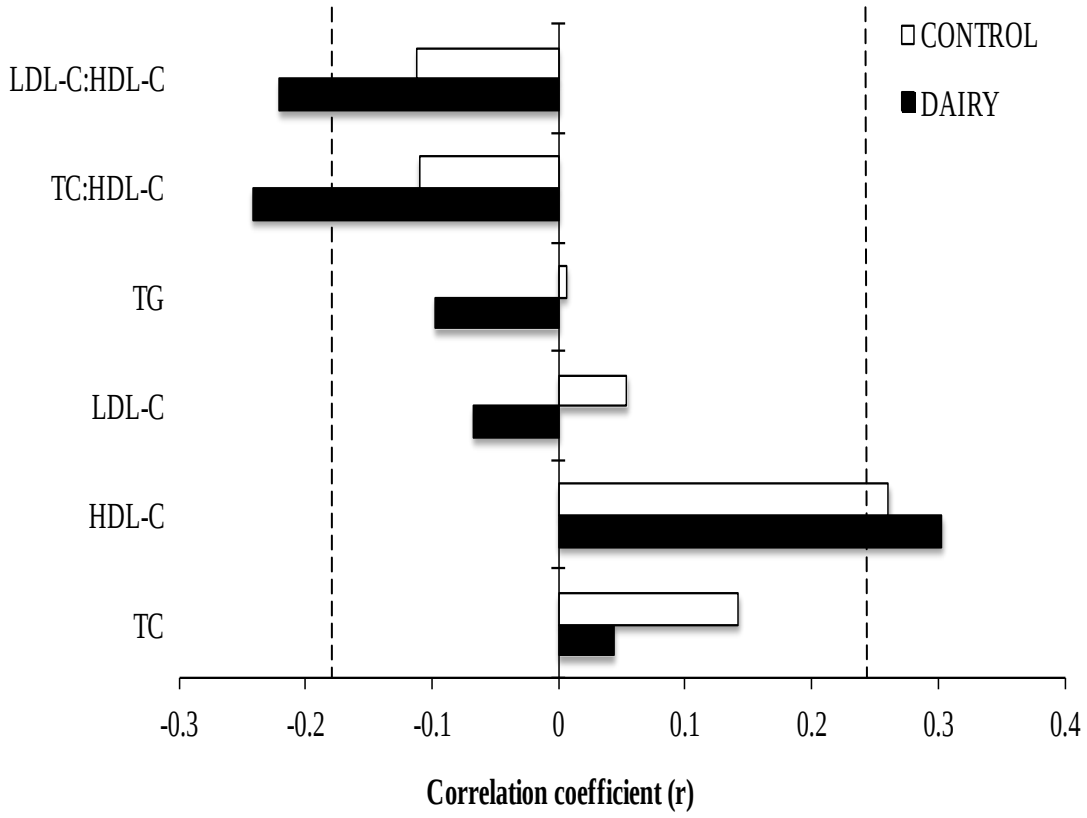

Figure 4.2 Relationships between plasma 25(OH)D and lipid levels at the end of each dietary phase (Spearman correlation coefficient data). CONTROL, energy-equivalent control products; DAIRY, commercial dairy products. Dash lines indicate significant associations ($P < 0.05$). LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

Table 4.6 Plasma fatty acid levels at the end of each dietary phase[1]

Fatty acid (% of total identified)	CONTROL		DAIRY		Δ (%)	*P*
	Mean	SE	Mean	SE		
C6:0	0.56	0.03	0.60	0.04	6.8	0.25
C8:0	0.19	0.01	0.20	0.01	3.3	0.66
C10:0	0.28	0.04	0.33	0.05	21.6	0.69
C12:0	0.15	0.01	0.15	0.01	−2.6	0.24
C14:0	0.68	0.02	0.70	0.02	2.8	0.12
C15:0	0.22	0.01	0.26	0.01	17.2	<0.0001
C16:0	28.84	0.20	29.07	0.20	0.8	0.14
trans-16:1n-7	0.26	0.01	0.25	0.01	−5.6	0.15
cis-16:1n-7	1.01	0.03	1.01	0.03	-0.2	0.64
C17:0	0.39	0.01	0.42	0.01	8.1	<0.0001
C18:0	11.36	0.14	11.32	0.14	−0.4	0.54
C18:1n-9	12.16	0.14	11.88	0.15	−2.4	0.03
cis-18:1n-7	1.57	0.03	1.49	0.02	−5.3	0.01
C18:2n-6	20.89	0.23	20.39	0.23	−2.4	0.03
C18:3n-6	0.16	0.01	0.18	0.01	9.7	0.03
C18:3n-3	0.48	0.02	0.48	0.02	−0.2	0.81
C20:0	0.38	0.01	0.39	0.01	1.5	0.51
cis-9,*trans*-11-18:2n-6	0.16	0.01	0.18	0.01	10.5	0.06
C20:1n-9	0.19	0.01	0.18	0.01	−7.1	0.01
C20:2n-6	0.32	0.01	0.31	0.01	−3.1	0.22
C20:3n-6	2.67	0.07	2.76	0.07	3.5	0.12
C20:4n-6	8.50	0.21	8.46	0.19	−0.5	0.83
C22:0	0.86	0.05	0.86	0.05	0.7	0.38
C20:5n-3	0.26	0.02	0.27	0.01	2.0	0.13
C22:1n-9	1.04	0.05	1.18	0.05	13.5	0.0006
C24:0	0.44	0.04	0.46	0.04	4.5	0.93
C22:4n-6	0.56	0.04	0.57	0.04	1.7	0.66
C24:1n-9	1.25	0.07	1.19	0.06	−4.5	0.43
C22:5n-3	1.21	0.05	1.26	0.05	3.5	0.15
C22:6n-3	3.93	0.12	3.85	0.12	−2.1	0.56
Total SFAs	43.59	0.18	44.13	0.21	1.3	0.003
Total MUFAs	17.28	0.18	17.07	0.16	−1.3	0.33
Total PUFAs	38.96	0.29	38.44	0.29	−1.3	0.24
Total n-6 PUFAs	33.22	0.24	32.66	0.26	−1.7	0.06
Total n-3 PUFAs	5.85	0.16	5.79	0.15	−1.1	0.85
n-6/n-3 ratio	5.99	0.16	6.00	0.15	0.2	1.00
MCFAs[2]	0.89	0.04	0.93	0.05	4.1	0.43

Table 4.6 *Continued*

Fatty acid (% of total identified)	CONTROL		DAIRY		Δ (%)	*P*
	Mean	SE	Mean	SE		
SFA/MUFA ratio	2.54	0.03	2.61	0.03	2.7	0.01
SFA/PUFA ratio	1.12	0.01	1.16	0.02	3.7	0.005
MUFA/PUFA ratio	0.45	0.01	0.45	0.01	1.0	0.93

CONTROL, energy-equivalent control products; DAIRY, commercial dairy products.
[1] Values are means ± SE after excluding outlier FA values based on mean ± 4 SDs; one FA value excluded from each of 18 FA categories (C6:0, C8:0, C12:0, C14:0, C15:0, C16:0, *cis*-16:1n-7, C18:1n-9, C18:2n-6, C18:3n-6, C20:0, C20:1n-9, C20:2n-6, C24:1n-9, total MUFAs, total PUFAs, MCFAs, and SFA/MUFA ratio), two FA values excluded from each of 10 FA categories (C10:0, *trans*-16:1n-7, *cis*-18:1n-7, C18:3n-3, C24:0, total SFAs, total n-6 PUFAs, n-6/n-3, SFA/PUFA, and MUFA/PUFA), and three FA values excluded from each of 2 FA categories (C20:5n-3 and C22:5n-3).[2] Sum of FAs C6:0-C12:0. n = 119 in CONTROL and n = 121 in DAIRY as plasma samples were missing for 5 and 3 participants in CONTROL and DAIRY, respectively. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; MCFA, medium chain fatty acid.

CONTROL) regardless of the levels of CRP at baseline. Intake of dairy products also resulted in higher plasma levels of total SFA ($P = 0.003$), C18:3n-6 ($P = 0.03$), and C22:1n-9 ($P = 0.0006$), as well as higher SFA/MUFA and SFA/PUFA ratios ($P = 0.01$ and 0.005, respectively), whereas levels of *cis*-18:1n-7, C18:1n-9, C18:2n-6, and C20:1n-9 were higher after CONTROL ($P < 0.05$ for all) (**Table 4.6**).

Other FAs of interest, including MCFAs (sum of C6:0-C12:0) and *trans*-16:1n-7 failed to show significant increases with DAIRY (0.93 ± 0.05 *vs.* 0.89 ± 0.04 % and 0.25 ± 0.01 *vs.* 0.26 ± 0.01 % of total identified FAs, $P = 0.43$ and 0.15, respectively). Except for MUFAs ($r = 0.17$, $P = 0.009$), there were no correlations detected between intakes of total FAs (i.e. SFAs, PUFAs, and MCFAs) as estimated by the FFQs and their corresponding FA levels in plasma (not shown). Correlations were, however, observed between dietary intakes of certain long chain PUFAs, such as C22:6n-3 and their levels in plasma ($r = 0.22$, $P = 0.0007$) as well as between dairy intake and plasma FAs C15:0 ($r = 0.27$, $P < 0.0001$) and C17:0 ($r = 0.25$, $P = 0.0002$).

4.5 DISCUSSION

This study assessed impacts of the recommended intake of commercially-available dairy products, specifically milk, yogurt, and cheese, in Canada on circulating FA and lipid profiles. In comparison to a dairy-free CONTROL prudent diet, a 4-week intake of 3 servings/day of commonly-consumed low-fat milk and yogurt products, and regular cheese resulted in higher plasma levels of the FAs C15:0, C17:0, C18:3n-6, C22:1n-9, and C22:5n-3. This was accompanied by a small yet statistically significant increase in serum LDL-C but not in other lipid levels.

Among classes of food groups, dairy is a subject of controversy when assessed for impacts on cardiovascular health, in part owing to its value as a source of certain FAs with established favourable and others with potential unfavorable implications (37, 38). Still, a gap continues to exist in research examining the degree to which dairy fat intake is reflected in the circulating FA profile, a modulator of lipid levels and hence CVD risk.

In agreement with our hypothesis and data from a collection of mainly long-term observational studies (18-20, 22, 24, 39-41), plasma FAs C15:0 and C17:0 levels were higher following DAIRY in the present work. These two odd-chain SFAs cannot be synthesized in the human body, and are specific to dairy fat and ruminant meat owing to actions of the microbiome in the rumen of bovine species (42), making them potential biomarkers of dairy intake. Some studies have already associated levels of C15:0 and/or C17:0 in circulation or adipose tissue with positive (22, 43) or negative (23) cardiovascular impacts. A 2014 meta-analysis of data from 4 cohort studies involving 5,490 participants showed an association between higher circulating C17:0 levels and 23% lower CHD risk (15). Adding to these longer-term observations, we here provide evidence on the reflection of FAs C15:0 and C17:0 in plasma to a short-term dairy fat intake.

Although the sum of plasma SFAs was higher after DAIRY, no significant between-treatment differences were observed in plasma levels of other individual SFAs that are naturally found in higher proportions in dairy, such as C14:0, C16:0, and C18:0. This is not surprising given the possible *de novo* synthesis of most SFAs from carbohydrates or their endogenous desaturation into MUFAs (44); circulating SFAs thus could but may not

necessarily reflect dietary SFA intake. The sum of plasma MUFA levels, on the other hand, was not different between the two treatments although plasma levels of certain MUFAs, such as C18:1n-9, were in particular higher in CONTROL, likely as a result of the intakes of cashews, which contain approximately 27 g C18:1n-9/100 g serving (45), and/or the endogenous synthesis from C18:0 via delta-9 destauration (44). Another FA of interest that did not show higher levels with DAIRY in the present work is *trans*-palmitoleic acid, the circulating levels of which have been shown to reflect whole-fat dairy consumption in recent cohort-based multivariate analyses (26). This lack of change is possibly due to our use of low-fat dairy products, except for the cheese (30 g/day), and the relatively short-term duration of intake. Additionally, *trans*-palmitoleate is not specific to dairy fat as it can also be principally derived from other ruminant fat sources or, further, synthesized in the body (46).

Plasma levels of three other individual FAs were also found to be higher after the 4-week intake of milk, yogurt, and cheese, including *cis*-9,*trans*-11-18:2n-6 (a trend), C18:3n-6, and C22:1n-9. An increase in the circulating levels of CLA was expected given that dairy products are natural sources of this FA, although there is a paucity of studies examining the association. This observation replicated those from a 2008 study, where circulating CLA contents increased in a dose-response manner with CLA supplementation (47). The increase in plasma levels of C18:3n-6 and C22:1n-9 with DAIRY in our study was not expected and can perhaps be attributed to the study's free-living design, and the presentation of individual FA values as standard percentages of total where certain FAs might have shown increases in compensation for decreases in other FAs. This possibility warrants verification via replication and mechanistic studies.

The absence of an increase in plasma levels of MCFA with DAIRY can be attributed to the fact that two of the three dairy products consumed had a low fat content. The unique metabolism of MCFA can also be considered to explain the lack of change in these FA plasma levels after DAIRY. During digestion, MCFAs bypass the chylomicron formation in the small intestine, directly entering the portal circulation for rapid and more complete oxidization in the liver (48). This is evident by data derived from some studies associating intakes of MCFA in oil formulas with increased fat oxidation (49) and energy expenditure (50), or decreased body fat mass (51). No study has, however, reported a reflection of dairy-specific MCFAs in plasma, calling for further investigation.

The observed slight but significantly higher serum LDL-C levels and LDL-C:HDL-C ratio after 4 weeks on DAIRY in comparison to CONTROL may in part be attributed to the reported higher intake of SFAs; 10.7 *vs.* 8.5% of total calorie intake ($P < 0.0001$), respectively. However, in absence of a correlation, it is more likely that higher intakes of certain foods, such as cashews, fruits, and vegetables, or nutrients, such as dietary fibre and phytosterols played a protective role during CONTROL. For instance, although not unambiguous or without exception (52), nuts have been shown to have cholesterol-lowering effects (53). In any case, considering the 0.08 mmol/L difference between the two treatments and Cohen's *d* value of 0.09 (54), the effect size on LDL-C is trivial and suggests no clinical significance as is further evidenced by the comparable FRSs at the end of each dietary phase. These observations support the majority of clinical trials in the literature, where dairy intakes show neutral effects on lipid profiles (55) and are in observational studies often associated with lower risk of CHD and all-cause mortality (56-58).

Our study has several strengths. It is the first and, to our knowledge, the largest crossover study examining impacts of the recommended 2-3 servings/day intake of certain dairy products, particularly in Canada, on circulating FA profile. The large number of participants increased our statistical power. The free-living design and use of various commercially-available, and widely-consumed, products mimicked the everyday diet of the general public, and are hence more relevant to a real life scenario. There are, however, some limitations. The study treatments were by nature different in more than one nutrient, which limited our potential ability to precisely identify the dietary factor(s) responsible for a given health effect. The reported between-treatment differences in serving/day of vegetables and fruit, and meat and alternatives food groups, although expected by study design, may limit the generalizability of our findings when considering that dairy can be replaced by other food products as well. The use of cashews during CONTROL may be seen as a limitation in terms of lipid evaluation. FFQs are susceptible to self-reported errors resulting in over- or under-estimations of intake. Still, the observed difference in dietary intake between DAIRY and CONTROL perfectly matched our experimental prescription, when statistical analyses on the food intake database that excluded products of the intervention indicated a close similarity in the participants' background diets during the two dietary phases (not shown). Data on circulating lipid levels are presented for the endpoint of each dietary phase based on a single blood sampling, however, given that lipid profiles naturally experience day-to-day fluctuations, a better estimation would have been achieved via repeated sampling and calculations of means. Also, a FA analysis of the study products would have been helpful when correlating dietary intake to circulating levels. These points support the future need for

fully-controlled clinical studies, and repeated laboratory assessments, to best evaluate the reflection of circulating FA and lipid profiles to a recommended dairy consumption. The role of genetic variations in influencing responses of these heath biomarkers to dairy intake is another fruitful area of research.

In conclusion, the present study provides evidence that the intake of 3 servings/day of conventional dairy products, particularly milk, yogurt, and cheese, in the context of a healthy-style diet, may modify the circulating FA profile in a manner that enables detectable changes in the cardio-protective FAs C15:0 and C17:0, with minor effect on lipid profile.

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the Dairy Farmers of Canada. MMHA, AC, MCL, and MEL have no conflict of interest to declare.

BL, PC, and PJHJ designed the study. PC was responsible for the screening and medical supervision of the study participants. MMHA, AC, and MCL coordinated the clinical trial and managed other aspects of the research. MMHA performed statistical analyses, interpreted clinical and laboratory data, and wrote the manuscript. MEL contributed to nutritional data and statistical analyses. BL and PJ had primary responsibility for final content. All authors critically reviewed the manuscript and approved its final version.

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BRIDGE TO CHAPTER V

In agreement with some but not all previous studies, Chapter IV has demonstrated that a blended dairy intervention may variably influence circulating cholesterol concentrations over a short term. Whether or not the degrees of such influence across individuals are modified through a genetic basis has not been investigated. Chapter V comprises a manuscript that, to our knowledge, for the first time reports a potential role of common variants of genes along cholesterol metabolic pathways in the responsiveness of circulating cholesterol to a recommended dairy intake.

CHAPTER V

MANUSCRIPT 3

This manuscript is under review in the

Journal of Nutrition.

COMMON VARIANTS IN CHOLESTEROL SYNTHESIS AND TRANSPORT RELATED GENES ASSOCIATE WITH THE EFFECT OF DAIRY INTAKE ON CIRCULATING CHOLESTEROL CONCENTRATIONS IN ADULTS

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5.1 ABSTRACT

Background: Dairy intake has been associated with varying impacts on circulating cholesterol concentrations across nutritional epidemiology and intervention studies, with findings attributed mainly to differences in the nature of dairy products or study designs. The contribution of the genomic architecture to such observations has yet to be revealed. **Objective**: To assess the relation of common single nucleotide polymorphisms in cholesterol-associated genes to serum cholesterol concentrations following recommended intakes of conventional dairy products. **Design**: In a multicentre randomized crossover design, 101 normolipidemic adults consumed 3 servings/day of DAIRY (1% milk fat (M.F.) milk, 1.5% M.F. yogurt, and 34% M.F. cheese) or energy-matched CONTROL products (fruit juice, vegetable juice, cashews, and cookies) provided within a prudent background diet for 4 weeks each, separated by a 4-8 week washout period. Serum lipid profile and genotyping analyses were completed by standard methods and TaqMan technology, respectively. **Results**: Overall, compared to CONTROL, DAIRY intake was associated with slightly elevated concentrations of total cholesterol (TC) (5.04 ± 0.13 *vs.* 5.15 ± 0.13 mmol/L, $P = 0.0296$) and LDL-cholesterol (LDL-C) (3.03 ± 0.10 *vs.* 3.13 ± 0.10 mmol/L, $P = 0.0179$). When separated based on genotypes, the cholesterol transport gene *ABCG5* rs6720173-G/G homozygotes responded to DAIRY with higher concentrations of TC (+0.18 mmol/L, $P = 0.0118$) and LDL-C (+0.17 mmol/L, $P = 0.0056$) relative to the C allele carriers (−0.07 and −0.06 mmol/L, respectively). Similarly, after DAIRY, the bile acid synthesis gene *CYP7A1* rs3808607-G allele carriers showed higher TC (+0.20 and +0.28 mmol/L for G>T and G/G genotypes, respectively, $P = 0.0026$) and LDL-C (+0.19 mmol/L for G>T genotype, $P = 0.0260$) compared with the

T/T homozygotes (−0.11 and −0.03 mmol/L for TC and LDL-C, respectively).

Conclusions: Genetic variations may contribute to differing associations between dairy intake and circulating cholesterol concentrations.

5.2 INTRODUCTION

An inter-individual variability has been documented in the reflection or association of circulating cholesterol concentrations, a classic biomarker of cardiovascular health, to nutrient intakes (1, 2) and attributed to interactions with the genetic background (3). To date the classical approach of these gene-nutrient interactions has been to explore single nutrients rather than whole foods. The latter are, however, typically better in capturing more accurate views into eating habits of populations and can thus aid in better tailoring of personalized dietary advice.

Dairy constitutes an array of food products with different nutritional compositions. Daily intake of these products is recommended in most food guides for optimal nutritional and thus overall health status. However, owing to their known nature as a rich source of dietary cholesterol and saturated fatty acids (SFAs), but also high-quality protein and several vitamins and minerals, dairy products are a subject of debate when assessed for associations with cardiovascular health. Whereas some epidemiological and dietary intervention studies report beneficial impacts of dairy intake on circulating cholesterol concentrations and/or cardiovascular disease (CVD) (4-7), other studies do not (8, 9). This inconsistency in research findings, underlined in a number of review articles (10-12) and meta-analyses (13), may have originated from differences in the dairy food matrix (14, 15), study designs (16), or simply other lifestyle and genetic factors.

In this regard, single nucleotide polymorphisms (SNPs) of certain genes have only recently been investigated for potential interactions with dairy intakes (17-19). For instance, apolipoprotein A2 (*APOA2*) SNP rs5082-C allele was associated with greater

BMI on a higher versus lower intake of high-fat dairy foods in 2 U.S. populations (18). Also, carriers of the peroxisome proliferator-activated receptor alpha (*PPARA*) SNP rs135549-T/T genotype, but not the C allele, had lower TC to HDL-C, and lower LDL-C to HDL-C ratios after a 12 mo intake of skim milk (19). No studies have, however, assessed the influence of common SNPs within genes that encode key metabolites in pathways of cholesterol homeostasis on an individual's responses of circulating cholesterol to dairy intake.

Given that dairy products are an integral part of most dietary guidelines, which recommend daily intakes of low- rather than high-fat dairy, and given that most nutrigenetic studies have to date focused on nutrients rather than whole foods, identifying genetic factors that may influence the impact of dairy-based diets on circulating cholesterol concentrations is warranted. Our objective was thus to test the hypothesis that heterogeneity within cholesterol-related genes would associate with the circulating cholesterol responses during intakes of conventional blended dairy in comparison to non-dairy products.

5.3 SUBJECTS AND METHODS

5.3.1 Study design and population

A multicentre randomized crossover trial was designed to investigate the impact of consuming the recommended concentration of dairy intake in North America, under free-living conditions, on established risk factors for CVD in a healthy Canadian population. Study methodology has been described in detail elsewhere (20, 21). Briefly, a nutritional

intervention of two 4-week phases, DAIRY and CONTROL, preceded by a 2-week run-in period and separated by a 4- to 8-week washout period, was conducted at the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN; University of Manitoba, Winnipeg) and the Institute of Nutrition and Functional Foods (INAF; Laval University, Québec City). The study protocol (www.clinicaltrials.gov; NCT01444326) was reviewed and approved by ethics boards of the two participating universities. As previously detailed (20, 21), over 600 individuals, aged 18-70 y, were screened and 212 found eligible based on the selection criteria. All study volunteers gave written informed consent upon enrolment.

5.3.2 Dietary protocols

At the run-in period, participants received nutritional advice emphasizing adherence to a prudent background dietary protocol throughout the study time and were provided with conventional dairy or energy-matched control products during weekly visits thereafter. Participants incorporated into their usual diets 3 servings/day of the study's dairy products, including 1% milk fat (M.F.) milk (375 ml), 1.5% M.F. creamy stirred fruit-flavoured yogurt (175 g), and 34% M.F. cheddar cheese (30 g), during the DAIRY phase. During the CONTROL phase, daily intake of the dairy products was replaced by 100% fruit juice (290 ml), 100% vegetable juice (156 ml), cashews (20 g), and a home-made cookie (39 g; prepared in the RCFFN and INAF metabolic kitchens). A summary of the study's dietary protocol and composition of food products was previously provided (20, 21).

5.3.3 Dietary assessment and compliance

Weekly checklists and validated web-based food frequency questionnaires (FFQs) (22), completed at the screening time and the end of each phase, were used to assess compliance to the dietary protocols. Intake of the study's dairy products was further confirmed by measuring the endpoint serum concentrations of 25(OH)D, a marker of vitamin D intake (21, 23).

5.3.4 Blood collection and serum lipid profile

Twelve-hour fasting venous blood samples were collected once at screening and twice within the last week of each dietary phase. Samples were centrifuged at 3000 rpm and 4°C for 20 min within 1-2 h of collection, and aliquots of blood fractions prepared and stored at −80°C until processing. Endpoint serum concentrations of TC, HDL-C, and TG from both study centres were determined in duplicates on a Roche/Hitachi Modular analyzer (Roche Diagnostics, Laval, QC, Canada). Serum LDL-C concentrations were estimated using the Friedewald formula (*LDL-C = TC - HDL-C - TG/2.2*) (24). Serum apolipoprotein B and plasma apolipoprotein B_{48} concentrations were measured by nephelometry with the Behring BN ProSpec (Siemens, Mississauga, ON, Canada) and a commercial ELISA kit (BioVendor, Asheville, NC, USA), respectively.

5.3.5 DNA extraction and genotyping

Genomic DNA was extracted from buffy coat samples using DNeasy® Blood & Tissue Kit (QIAGEN, Germantown, MD, USA). Concentration and purity of DNA were measured by NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Fisher Scientific,

Mississauga, ON, Canada). Genotyping of the cholesterol-related genes and SNPs of interest was completed using the TaqMan® GTXpress™ Master Mix with allele-specific probes on the StepOnePlus™ Real-Time PCR System (Applied Biosystems® Inc., Foster City, CA, USA). Polymorphisms in genes coding for well-established metabolites of cholesterol and bile acid synthesis, transport, and clearance were selected based on at least one of three criteria: possible functional effects (i.e. SNP in a missense region), higher minor allele frequency (MAF), and/or previously reported cholesterol-modulating gene-diet interactions. Twenty two SNPs within 13 genes including *ABCA1* (rs2230808, rs2066714), *ABCG5* (rs6720173, rs6756629, rs2278356), *ABCG8* (rs4148211, rs4148217, rs6544718), *ACAT2* (rs25683), *APOE* (rs7412, rs429358), *CETP* (rs5882), *CYP7A1* (rs3808607), *DHCR7* (rs760241, rs1044482), *LDLR* (rs688), *LSS* (rs2839158, rs34115287), *PCSK9* (rs562556), *SCAP* (rs12487736), and *SREBF2* (rs2228313, rs2228314) were assessed.

5.3.6 Statistical analyses

Statistical analyses were performed by SAS package version 9.2 (SAS Institute Inc., Cary, NC, USA). Abnormally distributed variables were natural log-transformed before analysis. Values were expressed as least-squares means (LS Means) and standard error of the mean (SEM), unless otherwise stated, with a $P < 0.05$ considered statistically significant. Dietary intakes during the various phases of the study were analyzed using non-parametric tests (Friedman test or Wilcoxon matched-pairs signed-rank test) and data were reported as medians and interquartile range (IQR). Lipid data at the end of DAIRY and CONTROL were compared by a PROC MIXED procedure for repeated measures,

with diet and gender as fixed effects, and study centre and subject as random effects. Gene-nutrient interaction data are presented as changes from endpoint CONTROL in response to endpoint DAIRY (delta) values and analyzed by analysis of covariance (ANCOVA), with genotype and gender as fixed effects, followed by Tukey-Kramer adjustments for multiple comparisons. When the number of the minor allele homozygotes failed to exceed 5% of the total population within a given SNP, they were combined with the heterozygotes of that SNP and compared to the major allele homozygotes. *APOE* isoforms, encoded by the two SNPs rs7412 and rs429358, and yielding 6 possible genotypes, are presented and compared as *APOE* E2 (sum of ε2/ε2, ε3/ε2, and ε4/ε2 genotypes), E3 (ε3/ε3 genotype), and E4 (sum of ε3/ε4 and ε4/ε4 genotypes).

5.4 RESULTS

5.4.1 Participant characteristics and compliance

One hundred and thirty-seven individuals were recruited, 124 completed the intervention, and 101 (72 females and 29 males), the data of whom are presented in this report, provided consent for genetic analyses (**Figure 5.1**). All participants were in good health and had average circulating lipid concentrations within acceptable ranges as defined by the *Canadian Cardiovascular Society Guidelines for the Diagnosis and Treatment of Dyslipidemia for the Prevention of Cardiovascular Disease in the Adult* (25) (**Table 5.1)**. Good compliance to the dairy treatment was evident by the self-reported 3.5 servings/day net difference in median intakes between dairy and control products ($P < 0.0001$) (**Table 5.2**), as well as by the higher ($P = 0.0004$) serum concentrations of 25(OH)D after DAIRY (80.7 ± 27.0 nmol/L) than after CONTROL (72.0 ± 29.0 nmol/L), similar to

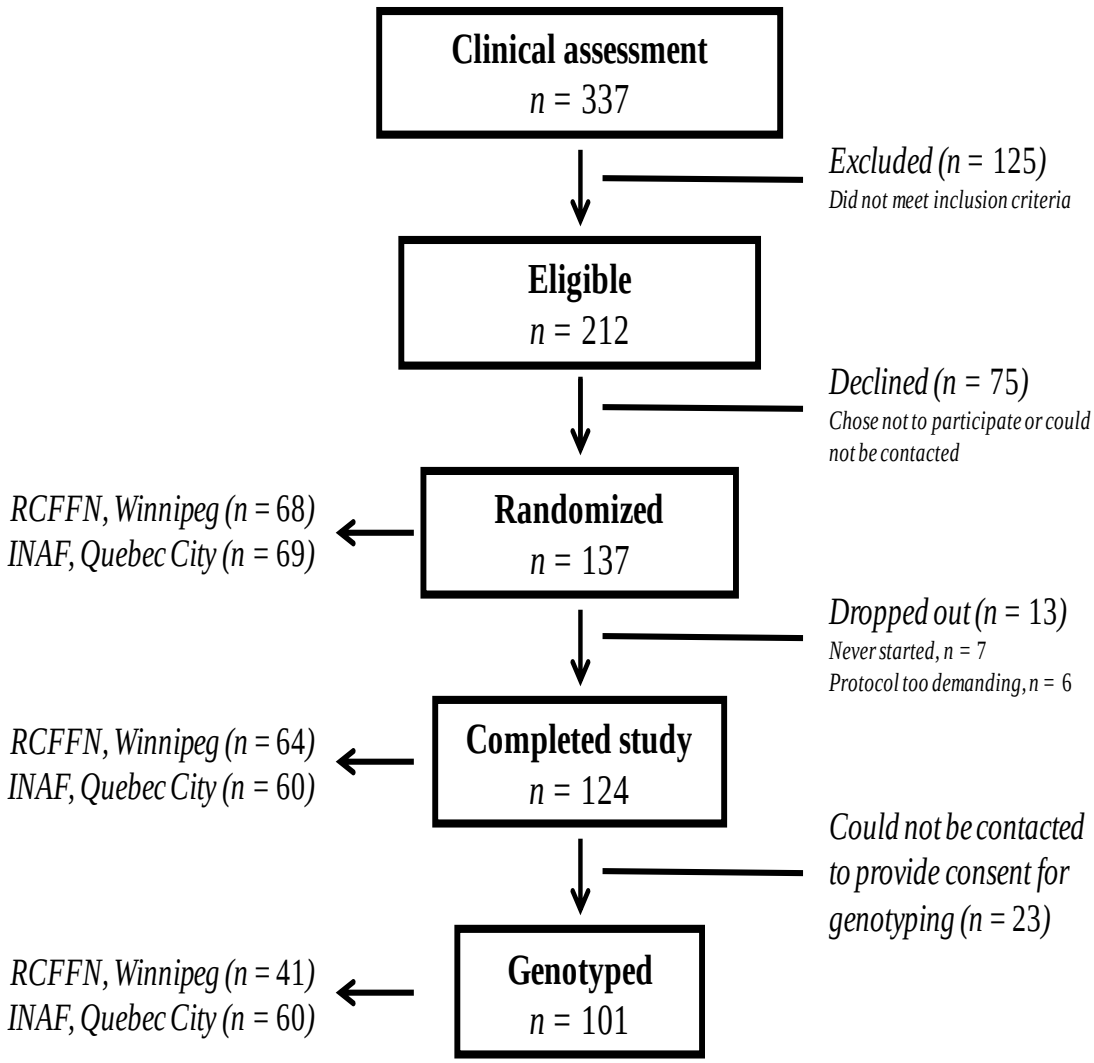

Figure 5.1 Flow chart of the study

Table 5.1 Baseline characteristics of the 101 study's participants[1]

	Mean ± SD[2]	Min-Max
Gender [n (%)]		
Men	29 (28.7)	
Women	72 (71.3)	
Ethnicity[3] [n (%)]		
Caucasian	75 (75)	
Asian	16 (16)	
African	3 (3)	
Hispanic	2 (2)	
Other	4 (4)	
Age (y)	41.7 ± 16.7	18-69
Anthropometry		
Body weight (kg)	71.7 ± 15.1	47.4-116.4
BMI (kg/m^2)	25.9 ± 4.3	17.8-36.4
Waist circumference (cm)	88.4 ± 13.2	63.0-115.3
Serum *hs*-CRP[4] (mg/L)	2.7 [2.5-2.9]	1.1-9.0
Plasma glucose[5] (mmol/L)	5.1 ± 0.4	4.4-6.1
Blood pressure[5] (mm Hg)		
Systolic	112.6 ± 13.1	83.7-148.0
Diastolic	66.7 ± 7.9	42.7-84.0
Plasma lipids[5] (mmol/L)		
TC	5.2 ± 1.0	3.1-7.9
HDL-C	1.7 ± 0.4	0.9-2.8
LDL-C	2.9 ± 0.9	1.2-5.3
TG	1.4 ± 0.7	0.4-3.2
TC/HDL-C ratio	3.3 ± 1.0	1.8-7.0
LDL-C/HDL-C ratio	1.9 ± 0.9	0.5-4.7
Baseline self-reported intakes by food groups[6] (servings/day)		
Dairy products	1.9 (1.8)	0.1-8.2
Fruits and vegetables	5.8 (3.6)	1.2-21.5
Grain products	4.5 (3.1)	1.2-11.6
Meat and alternatives	2.1 (1.7)	0.5-7.1

[1] Screening data. [2] Unless otherwise indicated. [3] Percent values based on $n = 100$ as data were missing for one participant. [4] Value is geometric mean [95% CI]. [5] Values are representatives of a subsample of 60 participants (Quebec Centre). [6] Values are medians (IQR) for $n = 99$ as dietary data from the FFQ were excluded for 2 participants because of non-plausible energy intakes (<500 or >3500 kcal in women and <800 or >4200 kcal in men). BMI, body mass index; HDL-C, HDL-cholesterol; *hs*-CRP; high sensitivity C-reactive protein; LDL-C, LDL-cholesterol; TG, triglycerides; TC, total cholesterol.

Table 5.2 Self-reported intakes by food groups and nutrients at the end of each dietary phase[1]

	CONTROL	**DAIRY**	***P***
Food group	*servings/day*		
Dairy products	0.0 (0.2)	3.5 (1.0)	<0.0001
Fruits and vegetables	8.7 (5.0)	5.7 (5.5)	<0.0001
Grain products	3.8 (2.7)	4.0 (2.2)	0.77
Meat and alternatives	2.6 (1.6)	2.0 (1.6)	<0.0001
Energy and nutrients			
Energy (kcal)	1759 (731)	1872 (731)	0.0072
Total fat (%)	32.7 (6.3)	30.2 (6.2)	<0.0001
SFA (%)	8.4 (2.1)	10.6 (2.2)	<0.0001
MUFA (%)	14.3 (3.4)	11.8 (3.1)	<0.0001
PUFA (%)	7.1 (1.7)	5.1 (1.7)	<0.0001
TFA (%)	1.1 (0.5)	1.1 (0.5)	0.42
Dietary cholesterol (mg)	174 (110)	211 (124)	<0.0001
Protein (%)	14.1 (3.2)	18.5 (3.4)	<0.0001
Total carbohydrate (%)	55.5 (7.6)	52.4 (7.7)	0.0001
Fibre (g)	24.4 (15.2)	21.2 (14.3)	0.0032
Sodium (mg)	2239 (834)	2311 (1103)	0.95
Calcium (mg)	437 (238)	141 (386)	<0.0001
Vitamin D (μg)	3.9 (4.4)	8.4 (5.5)	<0.0001

[1] Values are medians (IQR) for $n = 96$ in CONTROL, as dietary data from the FFQ were missing for 2 participants and excluded for 3 participants because of non-plausible energy intakes (<500 or >3500 kcal in women and <800 or >4200 kcal in men), and $n = 94$ in DAIRY, as dietary data were missing for 4 participants and excluded for 3 participants because of non-plausible energy intakes (same criteria). MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TFA, *trans* fatty acid.

what we have previously observed (21). As expected, intakes of fruits and vegetables, and meat and alternatives, were higher during CONTROL attributable to the intervention's specific control products ($P < 0.0001$).

Participants reported consuming more calories, SFA, dietary cholesterol, protein, calcium, and vitamin D, but less total fat, monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), total carbohydrates, and fibre during DAIRY incomparison to CONTROL ($P < 0.01$ for all) (**Table 5.2**).

5.4.2 Serum lipids

In comparison to CONTROL, DAIRY intake increased serum TC (5.04 ± 0.13 *vs.* 5.15 ± 0.13 mmol/L, $P = 0.03$) and LDL-C (3.03 ± 0.10 *vs.* 3.13 ± 0.10 mmol/L, $P = 0.02$) concentrations (**Table 5.3**). Whereas LDL-C/HDL-C ratio only showed a trend of higher value after DAIRY versus CONTROL (2.32 ± 0.10 *vs*. 2.27 ± 0.10, $P = 0.06$), other lipid fractions were not altered by the study treatments ($P > 0.10$).

5.4.3 Impact of individuals' genotypes on cholesterol concentrations

As in many controlled feeding trials a high inter-individual variability in the responses to the dietary intervention was observed in this study (**Figure 5.2**). Inter-individual variability in *ABCG5*, *CYP7A1*, and *DHCR7* genes was in particular shown to contribute to shaping the degree of serum TC and LDL-C responsiveness to DAIRY (**Tables 5.4** and **5.5**). Specifically, when expressed as absolute changes from CONTROL, TC and LDL-C concentrations were increased after DAIRY among the *ABCG5* SNP rs6720173-G/G homozygotes (+0.18 mmol/L ($P = 0.0118$) and +0.17 mmol/L ($P = 0.0056$),

Table 5.3 Serum lipid profile and electrophoresis characteristics of low-density lipoprotein particles at the end of each dietary phase[1]

	CONTROL	DAIRY	Δ (%)	*P*
Lipid profile				
TC (mmol/L)	5.04 ± 0.13	5.15 ± 0.13	2.25	0.0296
HDL-C (mmol/L)	1.46 ± 0.05	1.47 ± 0.05	0.85	0.53
TC/HDL-C ratio	3.70 ± 0.12	3.75 ± 0.12	1.28	0.12
TG (mmol/L)	1.22 ± 0.06	1.22 ± 0.06	0.00	0.58
APOB (g/L)	0.83 ± 0.03	0.85 ± 0.03	2.57	0.10
$APOB_{48}$ (ng/mL)	5660 ± 431	5651 ± 431	-0.16	0.24
LDL-C (mmol/L)	3.03 ± 0.10	3.13 ± 0.10	3.32	0.0179
LDL-C/HDL-C ratio	2.27 ± 0.10	2.32 ± 0.10	2.17	0.06
Relative distribution among LDL subclasses (%)				
LDL peak particle size (nm)	25.46 ± 0.65	25.48 ± 0.65	0.08	0.10
% Small (<25.5 nm)	51.77 ± 5.41	51.09 ± 5.41	-1.31	0.55
% Medium (25.5-26.0 nm)	30.44 ± 3.36	31.32 ± 3.36	2.91	0.14
% Large (>26.0 nm)	17.77 ± 2.08	17.57 ± 2.08	-1.11	0.35

[1] Values are least-square means (LS Means) ± SEMs. APOB, apolipoprotein B; $APOB_{48}$, apolipoprotein B_{48}; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; TG, triglycerides; TC, total cholesterol.

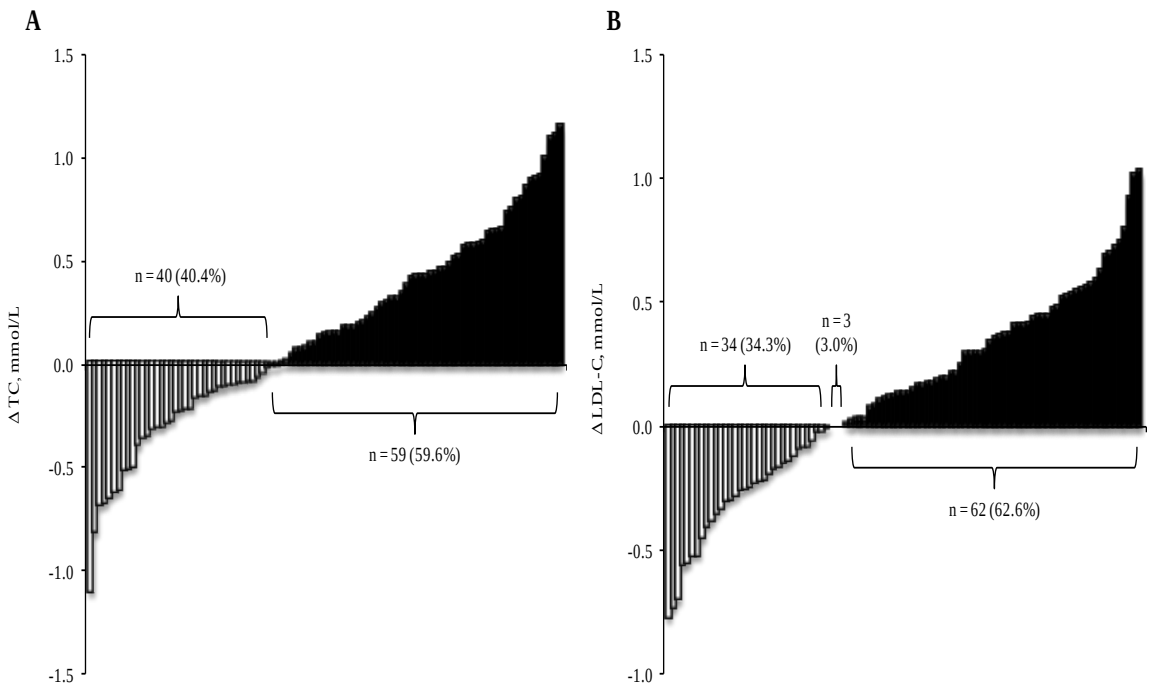

Figure 5.2 By-subject responses of serum total cholesterol (range: −1.10 to +1.15 mmol/L) (A) and LDL-cholesterol (range: −0.77 to +1.03 mmol/L) (B) concentrations to the DAIRY diet compared with CONTROL. Values are means for *n* = 99 as data for 2 participants were excluded because of non-plausible cholesterol differences between the study treatments based on *Cook's Distance* analysis. LDL-C, LDL-cholesterol; TC, total cholesterol; Δ, change in cholesterol (post-diet dairy minus control).

Table 5.4 Impact of single nucleotide polymorphisms within the cholesterol-related genes of interest on serum total cholesterol responses to DAIRY[1]

Gene	SNP	Genotype	*n*	Δ TC[2], mmol/L	Genotype *P*[3]
ABCA1	rs2230808	C/C	51	0.18 ± 0.07	
		C>T	38	0.01 ± 0.08	0.22
		T/T	10	0.14 ± 0.14	
	rs2066714	T/T	66	0.06 ± 0.06	0.12
		C>T + C/C	33	0.21 ± 0.08	
ABCG5	rs6720173	G/G	71	0.18 ± 0.06	0.0118
		C>G + C/C	28	−0.07 ± 0.07	
	rs6756629	G/G	90	0.12 ± 0.05	0.38
		A>G + A/A	9	−0.01 ± 0.15	
	rs2278356	A/A	36	0.24 ± 0.08	
		A>C	50	0.04 ± 0.07	0.12
		C/C	13	0.04 ± 0.12	
ABCG8	rs4148211	A/A	37	0.14 ± 0.08	
		A>G	42	0.07 ± 0.07	0.76
		G/G	20	0.13 ± 0.10	
	rs4148217	C/C	63	0.13 ± 0.06	0.56
		A>C + A/A	36	0.07 ± 0.08	
	rs6544718	C/C	60	0.13 ± 0.06	0.66
		C>T + T/T	39	0.08 ± 0.08	
ACAT2	rs25683	G/G	23	0.13 ± 0.10	
		A>G	46	0.09 ± 0.07	0.92
		A/A	30	0.12 ± 0.09	
APOE	rs7412 and rs429358	E2	15	0.02 ± 0.13	
		E3	63	0.15 ± 0.06	0.50
		E4	21	0.05 ± 0.10	
CETP	rs5882	A/A	42	0.18 ± 0.07	
		A>G	43	0.04 ± 0.07	0.37
		G/G	14	0.09 ± 0.12	
CYP7A1	rs3808607	T/T	33	−0.11 ± 0.08[a]	
		G>T	52	0.20 ± 0.06[b]	0.0026
		G/G	14	0.28 ± 0.12[b]	

Table 5.4 *Continued*

Gene	SNP	Genotype	*n*	Δ TC[2], mmol/L	Genotype *P*[3]
DHCR7	rs1044482	T/T	45	0.12 ± 0.07	
		C>T	35	0.16 ± 0.08	0.40
		C/C	19	−0.01 ± 0.11	
	rs760241	G/G	79	0.06 ± 0.06	0.0506
		A>G + A/A	20	0.28 ± 0.10	
LDLR	rs688	C/C	34	0.15 ± 0.09	
		C>T	50	0.08 ± 0.07	0.81
		T/T	15	0.13 ± 0.12	
LSS	rs2839158	C/C	71	0.07 ± 0.06	0.23
		C>T + T/T	28	0.19 ± 0.09	
	rs34115287	T/T	71	0.07 ± 0.06	0.20
		C>T + C/C	28	0.20 ± 0.09	
PCSK9	rs5622556	A/A	80	0.08 ± 0.06	0.29
		A>G + G/G	19	0.21 ± 0.11	
SCAP	rs12487736	T/T	31	0.06 ± 0.08	
		C>T	54	0.17 ± 0.07	0.23
		C/C	14	−0.04 ± 0.12	
SREBF2	rs2228313	G/G	90	0.11 ± 0.05	0.88
		C>G + C/C	9	0.09 ± 0.15	
	rs2228314	G/G	48	0.21 ± 0.07	
		C>G	38	0.03 ± 0.07	0.08
		C/C	13	−0.02 ± 0.13	

[1] Values are LS Means ± SEMs; $n = 99$ as data for 2 participants were excluded based on Cook's Distance analysis. [2] Values within a column with different superscript letters are significantly different, $P = 0.0052$ and 0.0176 for *CYP7A1* rs3808607-G>T and G/G, respectively. [3] ANCOVA and post hoc Tukey's test; adjusted for gender. *ABCA1*, ATP-binding cassette sub-family A (WHITE) member 1; *ABCG5*, ATP-binding cassette sub-family G (WHITE) member 5; *ABCG8*, ATP-binding cassette sub-family G (WHITE) member 8; *ACAT2*, acetyl-CoA acetyltransferase; *APOE*, apolipoprotein E; *CETP*, cholesteryl ester transfer protein; *CYP7A1*, cholesterol 7-alpha-hydroxylase; *DHCR7*, 7-dehydrocholesterol reductase; LDL-C, LDL-cholesterol; *LDLR*, low-density lipoprotein receptor; *LSS*, lanosterol synthase; *PCSK9*, proprotein convertase subtilisin/kexin type 9; *SCAP*, SREBF chaperone; *SREBF2*, sterol regulatory element binding transcription factor 2; TC, total cholesterol.

Table 5.5 Impact of single nucleotide polymorphisms within the cholesterol-related genes of interest on serum LDL-cholesterol responses to DAIRY[1]

Gene	SNP	Genotype	*n*	Δ LDL-C[2], mmol/L	Genotype *P*[3]
ABCA1	rs2230808	C/C	51	0.17 ± 0.06	
		C>T	38	0.04 ± 0.06	0.30
		T/T	10	0.11 ± 0.12	
	rs2066714	T/T	66	0.06 ± 0.05	0.08
		C>T + C/C	33	0.20 ± 0.07	
ABCG5	rs6720173	G/G	71	0.17 ± 0.05	0.0056
		C>G + C/C	28	−0.06 ± 0.07	
	rs6756629	G/G	90	0.11 ± 0.04	0.56
		A>G + A/A	9	0.04 ± 0.12	
	rs2278356	A/A	36	0.22 ± 0.07	
		A>C	50	0.06 ± 0.06	0.09
		C/C	13	0.02 ± 0.10	
ABCG8	rs4148211	A/A	37	0.15 ± 0.07	
		A>G	42	0.05 ± 0.06	0.41
		G/G	20	0.16 ± 0.09	
	rs4148217	C/C	63	0.13 ± 0.05	0.40
		A>C + A/A	36	0.06 ± 0.07	
	rs6544718	C/C	60	0.13 ± 0.05	0.40
		C>T + T/T	39	0.07 ± 0.06	
ACAT2	rs25683	G/G	23	0.11 ± 0.08	
		A>G	46	0.05 ± 0.06	0.28
		A/A	30	0.19 ± 0.07	
APOE	rs7412 and rs429358	*E2*	15	0.03 ± 0.11	
		E3	63	0.11 ± 0.05	0.73
		E4	21	0.12 ± 0.08	
CETP	rs5882	A/A	42	0.17 ± 0.06	
		A>G	43	0.07 ± 0.06	0.34
		G/G	14	0.03 ± 0.10	
CYP7A1	rs3808607	T/T	33	−0.03 ± 0.07[a]	
		G>T	52	0.19 ± 0.06[b]	0.0343
		G/G	14	0.12 ± 0.10[ab]	

Table 5.5 *Continued*

Gene	SNP	Genotype	*n*	Δ LDL-C[2], mmol/L	Genotype *P*[3]
DHCR7	rs1044482	T/T	45	0.12 ± 0.06	
		C>T	35	0.15 ± 0.07	0.27
		C/C	19	−0.02 ± 0.09	
	rs760241	G/G	79	0.06 ± 0.05	0.0399
		A>G + A/A	20	0.26 ± 0.08	
LDLR	rs688	C/C	34	0.14 ± 0.07	
		C>T	50	0.10 ± 0.06	0.76
		T/T	15	0.05 ± 0.10	
LSS	rs2839158	C/C	71	0.08 ± 0.05	0.31
		C>T + T/T	28	0.17 ± 0.07	
	rs34115287	T/T	71	0.08 ± 0.05	0.26
		C>T + C/C	28	0.18 ± 0.08	
PCSK9	rs5622556	A/A	80	0.09 ± 0.05	0.44
		A>G + G/G	19	0.17 ± 0.09	
SCAP	rs12487736	T/T	31	0.06 ± 0.07	
		C>T	54	0.16 ± 0.06	0.25
		C/C	14	−0.00 ± 0.10	
SREBF2	rs2228313	G/G	90	0.11 ± 0.04	0.94
		C>G + C/C	9	0.10 ± 0.13	
	rs2228314	G/G	48	0.18 ± 0.06	
		C>G	38	0.07 ± 0.06	0.13
		C/C	13	−0.05 ± 0.11	

[1] Values are least-square means (LS Means) ± SEMs; $n = 99$ as delta data for 2 participants were excluded based on *Cook's Distance* analysis. [2] Values within a column with different superscript letters are significantly different, $P < 0.0260$. [3] ANCOVA and post hoc Tukey's test; adjusted for gender. *ABCA1*, ATP-binding cassette sub-family A (WHITE) member 1; *ABCG5*, ATP-binding cassette sub-family G (WHITE) member 5; *ABCG8*, ATP-binding cassette sub-family G (WHITE) member 8; *ACAT2*, acetyl-CoA acetyltransferase; *APOE*, apolipoprotein E; *CETP*, cholesteryl ester transfer protein; *CYP7A1*, cholesterol 7-alpha-hydroxylase; *DHCR7*, 7-dehydrocholesterol reductase; LDL-C, LDL-cholesterol; *LDLR*, low-density lipoprotein receptor; *LSS*, lanosterol synthase; *PCSK9*, proprotein convertase subtilisin/kexin type 9; *SCAP*, SREBF chaperone; *SREBF2*, sterol regulatory element binding transcription factor 2.

respectively) compared to the C allele carriers (−0.07 and −0.06 mmol/L for TC and LDL-C, respectively). After DAIRY intake, TC concentrations were also higher among carriers of the *CYP7A1* rs3808607-G allele (+0.20 mmol/L for the G>T genotype (P = 0.0052) and +0.28 mmol/L for the G/G genotype (P = 0.0176)), in comparison to the rs3808607-T/T homozygotes (−0.11 mmol/L). Similarly, LDL-C concentrations were higher in *CYP7A1* rs3808607-G>T heterozygotes (+0.19 mmol/L, P = 0.0260) compared to the T/T homozygotes (−0.03 mmol/L). Further, in response to DAIRY, TC and LDL-C concentrations were higher in carriers of the *DHCR7* SNP rs760241-A allele (+0.28 mmol/L (P = 0.0506) and +0.26 mmol/L (P = 0.0399), respectively) relative to the rs760241-G/G homozygotes (+0.06 mmol/L for both TC and LDL-C concentrations).

Combination analyses revealed additive effects of the genetic variants that modified the TC and LDL-C responsiveness to DAIRY versus CONTROL (**Figure 5.3**). For instance, carriers of the *ABCG5* rs6720173-G/G genotype who also carried the *CYP7A1* rs3808607-G allele (n = 49) showed higher TC and LDL-C concentrations (+0.23 ± 0.06 mmol/L (P < 0.0001) and +0.20 ± 0.06 mmol/L (P = 0.0004), respectively) compared with carriers of the *ABCG5* rs6720173-C allele who carried the *CYP7A1* rs3808607-TT genotype (n = 11; −0.42 ± 0.13 and −0.30 ± 0.11 mmol/L for TC and LDL-C, respectively). Similarly, after DAIRY, carriers of the *ABCG5* rs6720173-G/G genotype + the *DHCR7* rs760241-A allele (n = 12) showed higher TC and LDL-C concentrations (+0.38 ± 0.13 mmol/L (P = 0.0052) and +0.33 ± 0.10 mmol/L (P = 0.0023), respectively) compared with carriers of the *ABCG5* rs6720173-C allele + the *DHCR7* rs760241-GG genotype (n = 20; −0.17 ± 0.10 and −0.16 ± 0.08 mmol/L for TC and LDL-C, respectively). Also, in response to DAIRY relative to CONTROL, carriers of the

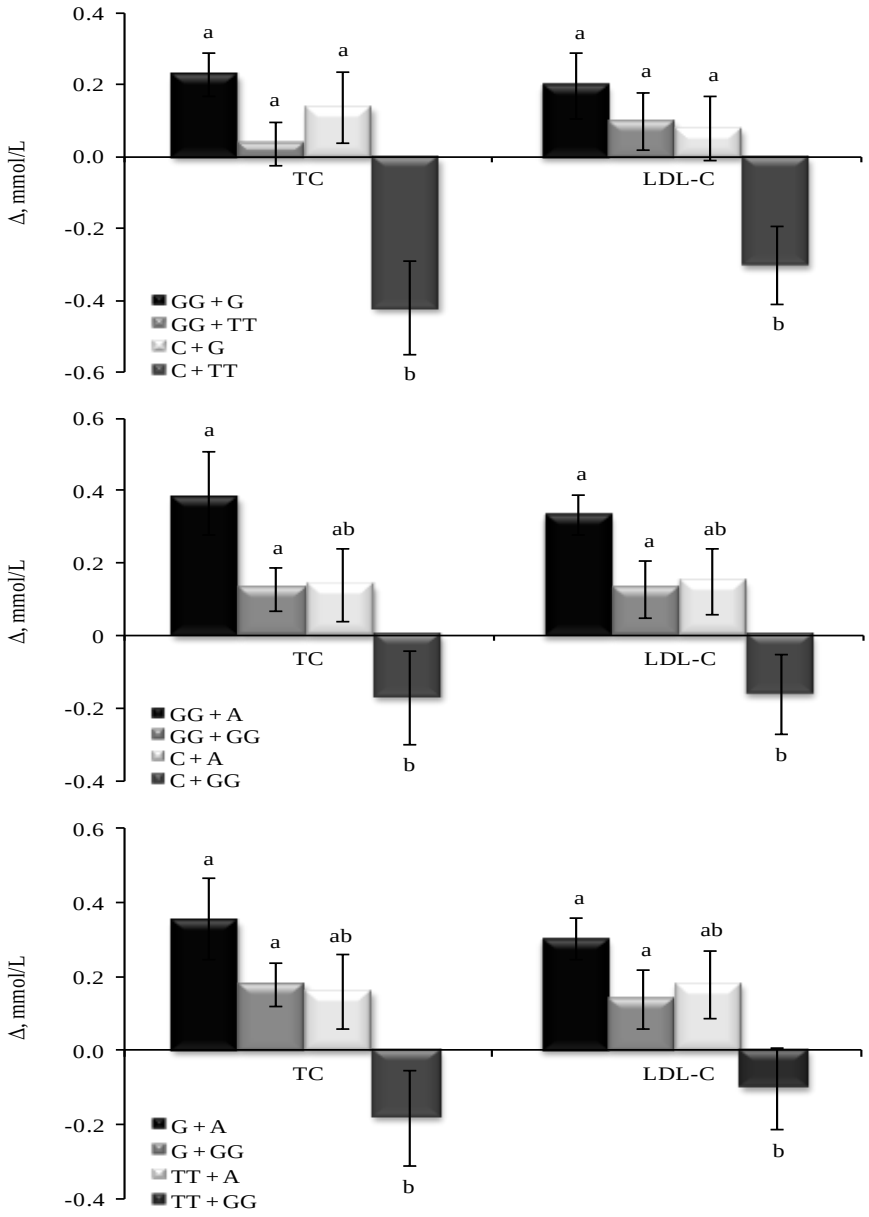

Figure 5.3 Responses of serum total cholesterol and LDL-cholesterol concentrations to DAIRY stratified by *ABCG5* rs6720173 + *CYP7A1* rs3808607 genotypes (A), *ABCG5* rs6720173 + *DHCR7* rs760241 genotypes (B), and *CYP7A1* rs3808607 + *DHCR7* rs760241 genotypes (C). Values are LS means ± SEMs. Effects of treatment by combined genotypes were assessed by using SAS ANCOVA, with gender as a fixed effect, and Tukey-Kramer adjustments. Labeled means without a common superscript letter are significantly different, $P < 0.05$. *ABCG5*, ATP-binding cassette sub-family G member 5; *CYP7A1*, cholesterol 7 α-hydroxylase; *DHCR7*, 7-dehydrocholesterol reductase; LDL-C, LDL-cholesterol; TC, total cholesterol; Δ, change in cholesterol (post-diet dairy minus control).

CYP7A1 rs3808607-G and *DHCR7* rs760241-A alleles (n = 13) showed higher TC and LDL-C concentrations (+0.35 ± 0.12 mmol/L (P = 0.0021) and +0.30 ± 0.10 mmol/L (P = 0.0113), respectively) compared with carriers of the *CYP7A1* rs3808607-TT and *DHCR7* rs760241-GG genotypes (n = 26; −0.18 ± 0.09 and −0.10 ± 0.07 mmol/L for TC and LDL-C, respectively).

5.5 DISCUSSION

The major finding of this work is that variations in key genes involved in regulation of cholesterol metabolism, specifically *ABCG5*, *CYP7A1*, and *DHCR7*, may modify the impact of conventional dairy product intake on serum lipid profile.

Many studies have investigated the influence of dairy product intake on circulating cholesterol concentrations as an established biomarker for cardiovascular health. Data have been mostly neutral and, when present, varied effects thought to relate to differences in the nature of dairy products consumed, study design, and subject phenotype (16, 26). Similar to our observations, some previous research showed an overall slightly higher TC and LDL-C concentrations after intakes of dairy diets (9, 27, 28), albeit with no statistical differences reported in most cases possibly due to the type of dairy product(s) employed and/or smaller sample sizes. For instance, a 21-week intake of 4 daily servings of dairy products in the forms of low-fat milk, cheese, and yogurt that provided a median of 1187 mg/day of dietary calcium, versus a control diet (median 691 mg/day calcium), failed to increase TC and LDL-C concentrations (0.1 and 0.2 mmol/L, respectively) (27). These findings disagreed with other old and recent reports showing hypocholesterolemic effects

of individual dairy products (29, 30), an inconsistency that may at least in part be explained by gene-nutrient interactions.

Only recently researchers have begun to investigate the genetic basis for the influence of dairy intakes on some health biomarkers of interest (17-19). The demonstrated increased TC and LDL-C responsiveness to DAIRY in this study was specifically associated with common variants in 3 genes; the major allele G homozygosity of the *ABCG5* SNP rs6720173 (MAF = 0.2106), the minor allele G of the *CYP7A1* SNP rs3808607 (MAF = 0.4496), and the minor allele A of the *DHCR7* SNP rs760241 (MAF = 0.1790) (**Tables 5.4** and **5.5**). To our knowledge this is the first nutrigenetic study to report differing responses of cholesterol concentrations to dairy product intake on the basis of genotypes in these particular genes.

The ATP-binding cassette sub-family G (WHITE) member 5 (ABCG5) is a half-transporter that, together with ABCG8, is responsible for cholesterol and plant sterol excretion from enterocytes into the intestinal lumen, and from hepatocytes into the bile (31). Presence of the G allele of SNP rs6720173 (1950 C>G), encoding a missense of amino acid 604Gln>Glu (Q604E), has not been previously associated with cholesterol concentrations in response to dietary cholesterol (32, 33), but to some extent with SFA intake (34). Weggemans et al. (32) reported a 13% higher "baseline" TC concentration among carriers of the G/G homozygotes (n = 13) versus those with the C allele (n = 473), but were unable to relate this effect to dietary cholesterol intake. In contrast, Herron et al. (33) found that plasma TC and LDL-C responses to dietary cholesterol (640 mg/day additional cholesterol) were actually higher among carriers of the C/C genotype than

among those with the G allele. Lastly, Viturro et al. (34) showed that, among Spanish children with 14.3-34.1 g/day SFA diet intake, only male carriers of the rs6720173-G allele had 9% and 19% higher plasma TC and LDL-C concentrations, respectively, in comparison to the C/C homozygotes. Mean intake of SFA in the DAIRY group of our study was 23 g/day (95% CI 21.5-24.7), which falls within the range reported in the latter study. No specific conclusion can be drawn on the basis of these mixed data, however, leaving a possibility for an overriding influence of other lifestyle or genetic factors, including other nutrients within dairy, other functional SNPs within the *ABCG5* gene, or interactions with other genes involved in cholesterol regulation. As pointed out earlier, these and the majority of studies in the literature examined individual nutrients rather than whole and complex foods such as dairy.

Cholesterol 7-alpha-hydroxylase (CYP7A1) is a rate-limiting enzyme for bile acid synthesis in the liver, the classical mechanism for cholesterol removal from the body (35). SNP rs3808607 (-204 T>G) is located in the promoter region (5' near gene) of the *CYP7A1* gene, where the ancestral and minor allele G has been proposed to have a higher transcriptional activity; in a plant sterol intervention this was thought to lead to an enhanced gene expression and increased bile acid synthesis (36). The findings of a potentially higher sensitivity of the *CYP7A1* SNP rs3808607-G allele towards the dietary protocol of our trial, leading to higher TC and LDL-C concentrations, are comparable with those from previous studies (37-39). For instance, among 131 Czech males whom food habits had changed over 8 years towards lower intakes of animal products and higher intakes of fruits and vegetables, homozygote carriers of the G allele showed a 25.5% reduction in plasma TC concentrations (change from baseline) compared to the T

allele carriers (37). Also, in a small crossover design with 11 healthy men (38), only the G/G homozygotes (n = 6) showed 15% and 38% higher serum TC and LDL-C concentrations, respectively, after a high-fat (40% en fat and 9.7 mg cholesterol/kg body weight per day) versus low-fat (22% en fat and 2.2 mg cholesterol/kg body weight per day) isocaloric diet intakes. There, no significant changes were observed in subjects who were homozygous for the T allele (n = 5). Further, among 209 normolipidemic subjects (39), the presence of the G allele was associated with ~63% higher plasma TC concentrations, when compared to the T/T genotype, after an increased intake of dietary cholesterol (742 ± 114 mg/day). The exact molecular mechanisms underlying these findings along with ours are as yet unknown. In theory, increased activity of *CYP7A1* would lead to increased bile acid synthesis rates and as a result decreased cholesterol concentrations in blood. One possibility, we speculate, relates to a compensatory decline in cholesterol excretion and/or a rise in cholesterol synthesis. In our study approximately 70% of the *CYP7A1* rs3808607-G allele carriers also carried the *ABCG5* rs6720173-G/G genotype. Indeed, combination analyses suggested an additive effect of these two variants with higher intakes of dairy.

Another genetically-oreinted impact of DAIRY in this study stemmed from an interaction with the *DHCR7* gene, which encodes 7-dehydrocholesterol reductase, the enzyme that catalyzes the terminal reaction in the cholesterol biosynthesis pathway. We are aware of no other gene-nutrient interactions in the literature associating SNP rs760241 in this gene to cholesterol concentrations. One possibility relates to a decresead activity of 7-dehydrocholesterol reductase among the A allele carriers upon intake of dairy diet, leading to lower biosynthesis rate of cholesterol from 7-dehydrocholesterol molecules.

However, this remains to be determined as activity of this enzyme was not measured and cholesterol concentrations are determined not only by this particular gene. Cholesterol concentrations in blood have been associated negatively with cholesterol synthesis, and positively with cholesterol absorption (40). Furthermore, a reciprocal interplay between cholesterol absorption and synthesis rates has been established (41, 42). Among the 20 subjects who were carriers of the *DHCR*7 rs760241-A allele in this study, 13 and 12 were also carriers of the *ABCG5* rs6720173-G/G genotype and the *CYP7A1* rs3808607-G allele, respectively. Combination analyses suggested additive effects of these variants on cholesterol concentrations, which, again, draws attention to the fact that gene-nutrient impacts are more likely to arise from a combination of functional polymorphisms on a given gene, or other genes of relevance, rather than a single polymorphism.

When assessed for impacts on circulating cholesterol, the majority of dairy-based interventions have thus far compared individual products, rather than studying dairy as a group. To our knowledge this is the first dairy study associating responses of circulating cholesterol concentrations with polymorphic variations in the *ABCG5*, *CYP7A1*, and *DHCR7* genes. We used dairy products that are commercially available and usually consumed by the general public, thus data from the study are generalizable to current practices in the population. The crossover design and the relatively large number of participants for such a dietary intervention represent other strengths. Also, unlike previous gene-nutrient studies that focused on a particular gene or SNP of a certain cholesterol-related function of interest, we genotyped several SNPs within a number of genes encoding metabolites from various aspects of cholesterol trafficking, including synthesis, transport, and excretion. The free-living setting, where the rest of the diet was

not controlled for, may be perceived as a limitation of this research, however. Although, there, efforts were undertaken to unify the background diet and ensure compliance to the dietary protocol across all individuals in the trial. In fact, our statistical analyses that excluded products of the intervention from the food intake database showed a similarity in the background diets of subjects during the two dietary phases (20).

In conclusion, the G/G genotype of *ABCG5* rs6720173 in 72% of the study population, the G allele of *CYP7A1* rs3808607 in 67% of the population, and the A allele of *DHCR7* rs760241 in 20% of the population, but not other genotypes, were specifically associated with higher serum TC and/or LDL-C concentrations after intakes of the study's dairy products. The clinical relevance of these observations is hard to predict and, given the small effect sizes, likely modest over short terms. Validation of the findings in future full-feeding interventions with larger sample sizes is thus necessary before dairy-based personalized nutritional recommendations may, on the long run, be implemented.

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The authors' responsibilities were as follows –MMHA, AC, and MCL: submission for ethical approvals, management of the clinical trial, and data collection; MMHA: laboratory analyses, data interpretation, statistical analyses, and drafting of the manuscript; PKE: genotyping expertise and assistance with the genetic analyses; PC:

medical supervision of the study subjects; PC, BL, and PJHJ: study concept and design; and BL and PJHJ: administrative, financial, and technical support, and study supervision. All authors contributed to critical reviews of the manuscript and approved its final version. The funding organizations had no role in defining the study design; in the collection, analysis and interpretation of data; in the writing of the manuscript; or in the decision to submit the manuscript for publication.

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BRIDGE TO CHAPTER VI

Cholesterol concentrations are known to be mediated by cholesterol metabolism through absorption in the intestine, *de novo* synthesis in the liver, and excretion through the bile. Using surrogate markers and stable isotope tracer techniques, the next chapter VI investigates the impact of the conventional dairy relative to non-dairy product intake on cholesterol metabolism, an aspect that has not been examined previously. After having demonstrated a genetically-orchestrated influence of dairy intake on circulating cholesterol concentrations in Chapter V, data of the manuscript in Chapter VI highlight, again for the first time, another gene-dairy interaction this time at the level of cholesterol absorption and synthesis rates.

CHAPTER VI

MANUSCRIPT 4

This manuscript is in submission to the

American Journal of Clinical Nutrition.

LOW-FAT AND REGULAR DAIRY PRODUCT CONSUMPTION MODIFIES [3, 4]^{13}C-CHOLESTEROL METABOLISM IN ASSOCIATION WITH GENETIC VARIANTS IN HEALTHY INDIVIDUALS

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6.1 ABSTRACT

Background: We have previously demonstrated associations between common variants in cholesterol-related genes and the degree of serum cholesterol responsiveness to dairy intake. Whether or not the presence of these variants modifies intestinal cholesterol absorption, hepatic biosynthesis, or biliary excretion is not known. **Objective**: We investigated the impact of a blended dairy intervention on cholesterol homeostasis as assessed through stable isotope tracers, surrogate markers, and gene-diet interaction studies. **Design**: In a randomized crossover study of two 4-week dietary phases, 40 healthy subjects consumed a prudent diet comprising 3 servings/day of low and regular fat milk, yogurt, and cheese (DAIRY) and a dairy-free CONTROL diet. Cholesterol absorption was determined by plasma campesterol and β-sitosterol concentrations, and by [3, 4]^{13}C-cholesterol enrichment over 96 hours following oral tracer administration. Cholesterol fractional synthesis rate (FSR) was measured by plasma lathosterol concentrations and by deuterium incorporation into RBC cholesterol over 24 hours. Single nucleotide polymorphisms (SNP) in 13 key genes related to cholesterol metabolism were assessed by TaqMan technology in a subgroup (n = 25). **Results**: DAIRY intake, relative to CONTROL, was associated with lower plasma campesterol (−8.0%, P = 0.017) and β-sitosterol (−19.0%, P < 0.0001) concentrations. Overall, no change in ^{13}C-cholesterol or FSR was observed between diets among the total study subjects. However, when separated on the basis of genotypes, after DAIRY intake, the major allele (T) homozygotes for the bile acid synthesis gene *CYP7A1* SNP rs3808607 showed lower ^{13}C-cholesterol enrichment (−325.3‰, P = 0.046), whereas the minor allele (A) carriers of the cholesterol synthesis gene *DHCR7* SNP rs760241 showed lower

FSR (−1.6 %/day, $P = 0.034$) in comparison to other genotypes. **Conclusions**: A recommended dairy consumption may modulate cholesterol homeostasis in individuals carrying specific genotypes within cholesterol-related genes.

6.2 INTRODUCTION

Atherogenic dyslipidemia, characterized by higher circulating concentrations of small LDL particles, together with lower HDL-cholesterol (HDL-C) and higher triglycerides (TG), is associated with increased risk of developing cardiovascular disease (CVD) (1). Indeed, CVD is reaching dramatic rates in Westernized countries with estimated economic burdens of CAD $12.1 billion in Canada (2), USD $315.4 billion in the U.S. (3), and about EU €196 billion a year in the European Union (4) representing total direct and indirect costs.

Features of dyslipidemia often stem from disturbances in cholesterol homeostasis (5), which is in turn mainly mediated by dietary and biliary cholesterol absorption in the intestine, *de novo* biosynthesis in the liver, and excretion through the bile (6, 7). As such, various methods have been developed for assessing the absorption and synthesis of cholesterol in humans (8). Stable isotope tracers (9) and the use of circulating phytosterol concentrations, such as campesterol and β-sitosterol, when expressed as ratios relative to total cholesterol (TC) (10), are established techniques for measurement of cholesterol absorption, whereas deuterium incorporation (11), and the measurement of circulating lathosterol to TC ratio (10), are known approaches for assessment of cholesterol biosynthesis. Additionally, circulating proprotein convertase subtilisin/kexin-9 (PCSK9) concentrations have in recent years been shown to predict the intra-cellular degradation rates of the LDL receptors, hence act as a maker of LDL particle clearance (12).

Dairy products, the specific consumption of which is recommended by food guides in many developed countries (13-16), have been repeatedly associated with varying impacts

on dyslipidemia (17-19) and CVD (20-23) in epidemiological and experimental studies alike. The disparities in the cholesterolemic impacts of dairy are in particular likely attributed to differences in the amount and type of products consumed, or simply in the designs and populations of studies. In any case, a paucity of evidence still exists as to whether, and if so to what extent, a recommended level of dairy product consumption alters cholesterol homeostasis. In this regard, a recent fully-controlled crossover study by our group, involving healthy postmenopausal women observed lower plasma phytosterol concentrations, a surrogate marker of cholesterol absorption, after intakes of 3.2 servings/day of milk per 2000 kcal than after a control NCEP milk-free diet (24).

On the other hand, we and others have previously demonstrated associations between certain genetic variants of common single nucleotide polymorphisms (SNP) within cholesterol-related genes and various degrees of individual circulating cholesterol responses to dietary intakes (25). Whether the presence of such variants determines responses of cholesterol homeostasis to dairy intakes is, however, not known.

The primary objective of this study was therefore to investigate the impact of dairy product consumption, as part of a prudent diet, on cholesterol homeostasis as assessed through surrogate markers and stable isotope tracer techniques. The secondary objective was to examine if genetic variations in key genes involved in the metabolic pathways of cholesterol influence the responses of cholesterol homeostasis to the dairy intervention. We hypothesized that dairy consumption modulates measures of cholesterol homeostasis in carriers of certain genotypes, but not others, within the study's cluster of genes of interest.

6.3 SUBJECTS AND METHODS

6.3.1 Subjects

The study methodology has been described in detail previously (26, 27). Briefly, men and women from the Winnipeg and Quebec City metropolitan areas were recruited using newspaper and radio advertisements, e-newsletters, and poster distribution. The study took place in 2011 concurrently at the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN; University of Manitoba) and the Institute of Nutrition and Functional Foods (INAF; Laval University). Deemed potentially eligible based on phone screenings, apparently healthy individuals between the age of 18 and 70 years with 1.0-10.0 mg/L C-reactive protein (CRP) concentrations, a marker of CVD, were recruited if they were free of monogenic dyslipidemia, endocrine, gastrointestinal, or any metabolic disorder, and were not routinely taking lipid-lowering, anti-hypertensive, or anti-inflammatory medications, or any nutritional supplements. Smokers, pregnant women, and individuals with dietary restrictions were also excluded from the study. The study protocol received approvals from ethics boards of the participating academic institutes and was registered on Clinicaltrials.gov **(**identifier# NCT01444326). All subjects signed informed consent prior to participating in the study.

6.3.2 Study design and protocol

The study was carried out as a multicentre free-living randomized crossover design where subjects followed a recommended prudent background dietary protocol with or without 3 servings/day of dairy products for a total duration of 12-16 weeks, including two 4-week

(28 days) dietary phases (DAIRY and CONTROL) and a 4-8 weeks washout period (median of 31 days). Dairy and "control" products were provided to the subjects on a weekly basis. During the DAIRY phase subjects consumed 375 ml/day of 1% milk fat (M.F.) milk, 175 g/day of 1.5% M.F. creamy stirred fruit-flavoured yogurt, and 30 g/day of 34% M.F. cheddar cheese, while in the CONTROL phase they consumed 290 ml/day of 100% fruit juice, 156 ml/day of 100% vegetable juice, 20 g/day cashews, and one 39 g/day RCFFN- and INAF-made cookie. The experimental products provided the same amounts of calorie; details on the study dietary protocol and nutritional compositions of products were previously published (26, 27).

On day 24 of each dietary phase, a random subsample of the study subjects at the RCFFN ingested 75 mg of the stable carbon isotope [3, 4]^{13}C-cholesterol (Cambridge Isotope Laboratories Inc., Tewksbury, MA, USA), mixed in 5 g of margarine and spread on a half of an English muffin, to measure cholesterol absorption over the following 72 hrs (days 25-28). Prior to breakfast on day 27 of each dietary phase, as well, an oral dose of 0.7 g/kg body water (estimated at 60% of total body weight) of deuterium oxide (^{2}H) (Cambridge Isotope Laboratories Inc., Tewksbury, MA, USA) was given orally to each of those subjects to measure endogenous cholesterol synthesis over the following 24 hrs. Fasting blood samples were collected at baseline (day 24) prior to the isotope administration as well as on days 25, 26, 27, and 28 to monitor the enrichment of both isotopes.

6.3.3 Dietary intakes

To monitor compliance to the dietary protocol, subjects completed weekly checklists and a validated web-based food frequency questionnaire (FFQ) (28) at the beginning of the study (screening time) and the end of each phase. Serum concentrations of 25-hydroxyvitamin D (25(OH)D) were also measured for the same purpose (29).

6.3.4 Blood collection and lipid profile

Twelve-hour fasting blood samples were collected at screening and on days 24 through 28 of each dietary phase, centrifuged at 3000 rpm and 4°C for 20 min, then made into aliquots of serum, plasma, red blood cells (RBC), and buffy coat (white blood cells; WBC) fractions, and stored at −80°C until analysis. Serum TC, HDL-C, and TG concentrations were measured in duplicates simultanously on a Roche/Hitachi Modular analyzer (Roche Diagnostics, Laval, QC, Canada). LDL-cholesterol (LDL-C) concentrations were calculated according to Friedewald formula (30).

6.3.5 Determination of surrogate markers of cholesterol homeostasis

Plasma campesterol, β-sitosterol, and lathosterol concentrations were assessed at the end of each dietary phase by a gas chromatography (GC), as described previously (31). The sum of campesterol and β-sitosterol concentrations is expressed in this article as total phytosterols. All values of these non-cholesterol sterols are presented in mg/L as well as in ratios relative to plasma total cholesterol concentrations (10^2 μmol/mmol TC) (32). Plasma PCSK9 concentrations, an indicative of the intra-cellular degradation rates of the LDL receptors in many tissues, were measured by a commercially available ELISA kit

(MBL International Corporation, Woburn, MA, USA).

6.3.6 Determination of cholesterol kinetics

Cholesterol absorption. Cholesterol absorption was determined at the RCFFN using the stable isotope single-tracer approach, as previously (33, 34). The lipid fraction of the RBCs collected on days 24-28 of each dietary phase was isolated using a modified Folch method (35). Lipid extracts were then analyzed by an on-line GC isotope ratio mass spectromety (GC-IRMS, ThermoFinnigan, Bremen, Germany) technique, where samples were run through a GC unit (Agilent 6890N; Agilent Technologies Canada Inc., Mississauga, ON, Canada**),** a combustion reactor (Combustion Interface III; Bremen, Germany), and a mass spectrometer (MS). In brief, lipids extracted were dissolved in hexane and separated by GC using a SAC-5 sterol column (Supelco, Milton, ON, Canada). Isolated free cholesterol was directed to the combustion reactor to release CO_2 into the MS, where the [3, 4]^{13}C enrichment of CO_2 ($\delta\ ^{13}CO_2/^{12}CO_2$) was determined relative to the reference gas and expressed relative to the international standard Vienna Pee Dee Belemita (V-PDB) limestone; isotope abundance was thus expressed in delta (δ) per mil (‰). The ^{13}C-cholesterol enrichments were measured for each of hours 0, 24, 48, 72, and 96 and area under the curve corrected for baseline values ($AUC_{24\text{-}96\ hr}$) was estimated, to represent cholesterol absorption in DAIRY and CONTROL, with the use of trapezoidal rule, as previously (36).

Cholesterol synthesis. Cholesterol synthesis was determined after 24 hour of deuterium water administration using the rate of deuterium incorporation within the body water pool into newly synthesized RBC free cholesterol pool, as previously described

(37). Lipid fraction of the RBCs collected on days 27 (hour 0) and 28 (hour 24) of each dietary phase were extracted using the same methods as with cholesterol absorption, and deuterium enrichments were completed by an on-line GC/pyrolysis/IRMS as described above. In brief, lipid extracts from RBCs were separated by a GC technique and free cholesterol was introduced into a pyrolysis reactor to release H_2 gas as described above. Plasma water samples were run through a high temperature conversion elemental analyzer (TC-EA). Deuterium enrichments for both RBC and plasma water samples were measured by IRMS relative to a reference gas (H_2). Normalization to Vienna standard mean ocean water (VSMOW) was performed using a regression equation between the on-line and off-line method (as in cholesterol absorption determination above) with data from the off-line method expressed relative to V-SMOW. The fractional rate of synthesis (FSR) for cholesterol was then calculated using the following equation:

$$\text{FSR (pools/day)} = \delta \text{ cholesterol}/(\delta \text{ plasma water} \times 0.478)$$

Where δ cholesterol is the difference in deuterium enrichment between hour 0 (day 27) and hour 24 (day 28), δ plasma water is the difference in deuterium enrichment between these hours for plasma water, and 0.478 is the ratio of labeled H atoms replaced by deuterium during *in vivo* biosynthesis.

6.3.7 DNA extraction and genotyping

Genomic DNA was extracted from WBC samples using the DNeasy® Blood & Tissue Kit (QIAGEN, Germantown, MD, USA), and assessed for quantity and purity by the NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Fisher Scientific Inc., Waltham,

MA, USA). Genotyping was completed by TaqMan® assay on the StepOnePlus™ Real-Time PCR System (Applied Biosystems® Inc., Foster City, CA, USA). Polymorphisms in genes of interest were selected on the basis of one or more of the following criteria: higher functionality, higher minor allele frequency (MAF), and reported cholesterol-influencing gene-diet interactions. Twenty two SNPs within 13 cholesterol metabolism-related genes were assessed, including rs2230808 and rs2066714 (*ABCA1*); rs6720173, rs6756629, and rs2278356 (*ABCG5*); rs4148211, rs4148217, and rs6544718 (*ABCG8*); rs25683 (*ACAT2*); rs7412 and rs429358 (*APOE*); rs5882 (*CETP*); rs3808607 (*CYP7A1*); rs760241 and rs1044482 (*DHCR7*); rs688 (*LDLR*); rs2839158 and rs34115287 (*LSS*); rs562556 (*PCSK9*); rs12487736 (*SCAP*); and rs2228313 and rs2228314 (*SREBF2*). *APOE-ε* genotypes, encoded by the two SNPs rs7412 and rs429358, were expressed as *APOE2/E2*, *E2/E3*, and *E2/E4* (*APOE*2), *E3/E3* (*APOE3*), and *E3/E4* and *E4/E4* (*APOE4*).

6.3.8 Statistical analyses

Data are reported as least-squares means (LSM) and SEM unless stated otherwise. The main analysis compared values of study outcomes at the end of DAIRY and CONTROL. Dietary intakes were analyzed using Friedman or Wilcoxon matched-pairs signed-rank tests, and were expressed as medians and interquartile range (IQR). Circulating lipids and surrogate markers of cholesterol homeostasis were analyzed using the PROC MIXED procedure for repeated measures, with diet and sex as fixed factors, and study centre as a random factor, followed by Tukey adjustments. Gene-diet interaction data were assessed using the analysis of covariance (ANCOVA) procedure, with genotype and sex as fixed

effects, followed by Tukey adjustments for multiple comparisons; these data are expressed as absolute changes from CONTROL in response to DAIRY (delta values). Minor allele homozygotes when less than 5% in a given SNP were merged with the heterozygotes and compared to the major allele homozygotes. Variables with a skewed distribution, as detected by Shapiro-Wilk test, were $\log_{10}$ transformed before statistical analysis. Associations between variables measured post treatment were determined by Pearson's or Spearman's Correlation Coefficients analyses depending on the normality of the variables. SAS software (version 9.2; SAS Institute Inc., Cary, NC, USA) was used for all analyses with a statistical significance set at $P < 0.05$.

6.4 RESULTS

6.4.1 Subject characteristics and dietary compliance

From both locations of the study, 137 individuals were recruited and 124 completed the dietary intervention, as detailed previously (26, 27). A random sample of 40 subjects from the RCFFN, the data of whom are presented in this report, completed all the study analyses in addition to the cholesterol kinetics, and 25 of those subjects were genotyped (**Figure 6.1**). Subject characteristics at the screening visit are shown in **Table 6.1**. The 40 individuals participating in this facet of study were fairly representative of all subjects in the study on the basis of age, BMI, and waist circumference (not shown). Compliance to the dairy treatment was evident by the self-reported median intake of 3.8 servings of dairy/day during DAIRY versus 0.0 servings dairy/day during CONTROL ($P < 0.0001$) (**Table 6.2**) and by the higher serum concentrations of 25(OH)D (72.6 ± 7.9 *vs.* 68.0 ± 7.9 nmol/L, $P = 0.0143$) after DAIRY than CONTROL. Calorie intake was similar under

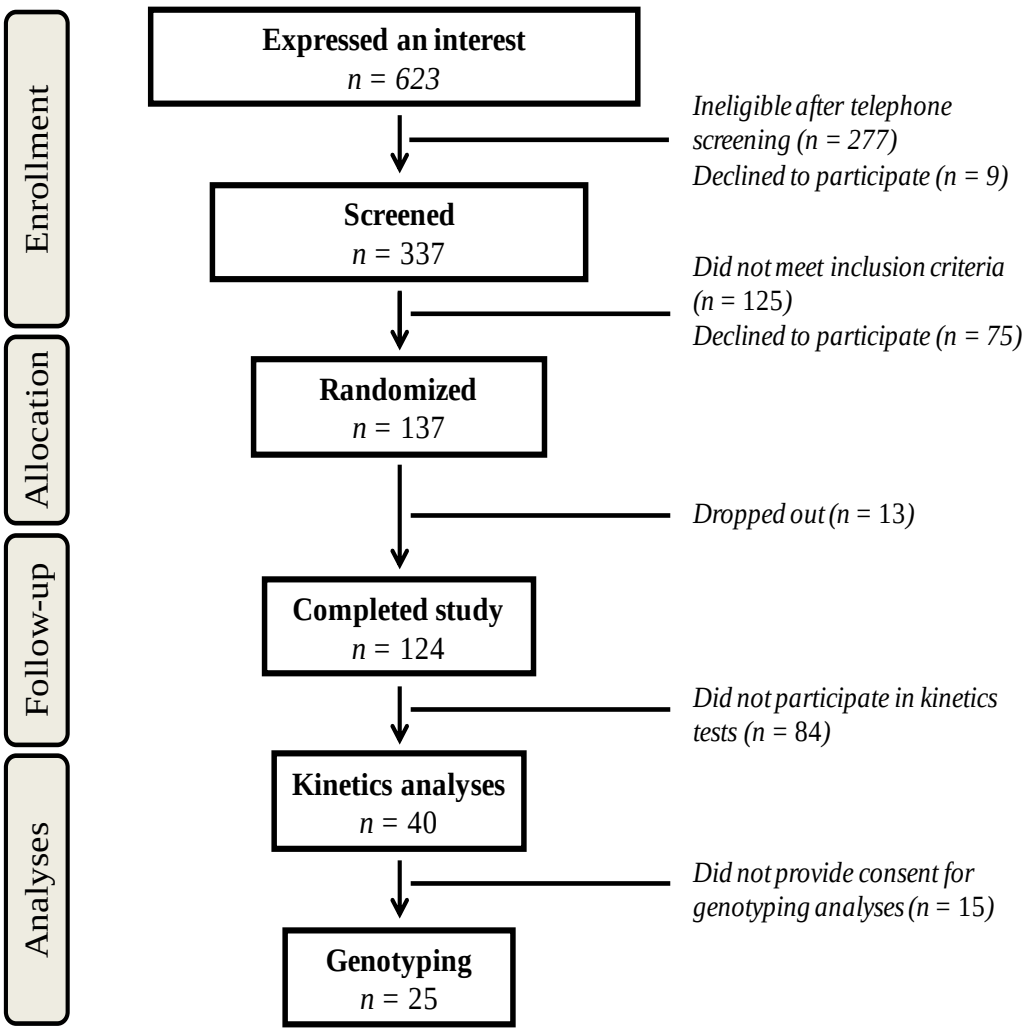

Figure 6.1 Consolidated Standards of Reporting Trials flow diagram.

Table 6.1 Subject characteristics at the screening visit

	Mean ± SD[1]	**Range**
Sex [n (%)]		
Men	17 (42.5)	
Women	23 (57.5)	
Ethnicity [n (%)]		
Caucasian	22 (55)	
Asian	14 (35)	
African	2 (5)	
Hispanic	1 (2.5)	
Other	1 (2.5)	
Age, *y*	39.5 ± 16.1	18-69
Anthropometry		
Body weight (kg)	74.8 ± 15.5	46.4-107.2
BMI (kg/m^2)	25.7 ± 4.4	17.4-36.3
Waist circumference (cm)	86.1 ± 14.4	61.5-118.0
Serum *hs*-CRP (mg/L)	2.7 ± 1.2	1.1-5.8
Baseline self-reported intakes by food groups[2] *(servings/day)*		
Dairy products	1.7 (2.2)	
Fruits and vegetables	6.5 (5.2)	
Grain products	3.7 (3.9)	
Meat and alternatives	2.2 (2.3)	

[1] Unless otherwise indicated. [2] Values are medians (IQR) for $n = 36$ as dietary data from the FFQ were excluded for 4 subjects because of non-plausible energy intakes (<500 or >3500 kcal in women and <800 or >4200 kcal in men). BMI, body mass index; *hs*-CRP; high sensitivity C-reactive protein.

both dietary treatments, however, the number of servings/day of fruits and vegetables ($P < 0.0001$), and meat and alternatives ($P = 0.0347$) were higher during CONTROL, as expected due to the nature of products provided during that phase. Intakes of SFAs, dietary cholesterol, protein, calcium, and vitamin D ($P < 0.01$ for all) were higher during DAIRY, whereas those of MUFA, PUFA, total carbohydrates, and fibre were higher during CONTROL ($P < 0.05$ for all) (**Table 6.2**).

6.4.2 Serum lipids and surrogate markers of cholesterol homeostasis

As shown in **Table 6.3**, the consumption of DAIRY did not significantly modify serum lipid concentrations ($P > 0.20$). Surrogate markers (mg/L) of intestinal cholesterol absorption were, however, significantly lower following the DAIRY phase (phytosterols −14%, $P < 0.0001$; campesterol −8.0%, $P = 0.017$; and β-sitosterol −19.0%, $P < 0.0001$) in comparison to CONTROL. A similar trend was observed when these markers were expressed in 10^2 μmol per mmol TC (**Table 6.3**). Concentrations (in mg/L and in 10^2 μmol per mmol TC) of plasma lathosterol ($P > 0.66$), a surrogate marker of endogenous cholesterol synthesis, and PCSK9 concentrations (−2.4%, $P = 0.66$), a regulator of LDL receptor degradation, were not different between the two diets (**Table 6.3**). Comparable findings of these measures were observed in the 25 individuals who were genotyped (not shown).

Table 6.2 Self-reported intakes by food groups and nutrients at the end of each dietary phases[1]

	CONTROL	**DAIRY**	***P***
Food group	*servings/day*		
Dairy products	0.0 (0.3)	3.8 (1.3)	<0.0001
Fruits and vegetables	8.7 (4.9)	4.8 (5.2)	<0.0001
Grain products	3.5 (4.3)	3.6 (3.2)	0.74
Meat and alternatives	2.6 (2.5)	2.3 (2.1)	0.0347
Energy and nutrients			
Energy (kcal)	1903.7 (993.6)	1896.3 (830.2)	0.37
Total fat (%)	33.8 (7.9)	30.7 (5.9)	0.25
SFA (%)	8.8 (2.1)	11.0 (2.2)	<0.0001
MUFA (%)	13.8 (4.4)	11.9 (2.7)	0.019
PUFA (%)	7.4 (1.9)	5.4 (2.0)	0.0002
TFA (%)	1.1 (0.8)	1.1 (0.7)	0.54
Dietary cholesterol (mg)	190.5 (189.9)	235.2 (174.1)	0.007
Protein (%)	13.9 (5.0)	18.1 (3.1)	<0.0001
Total carbohydrate (%)	55.9 (10.8)	51.8 (9.6)	0.0008
Fibre (g)	23.1 (25.2)	20.6 (17.4)	0.021
Sodium (mg)	2326.0 (1585.2)	2416.9 (1262.3)	0.67
Calcium (mg)	436.7 (336.3)	1515.6 (507.1)	<0.0001
Vitamin D (μg)	3.8 (6.9)	9.2 (8.0)	<0.0001

[1] Values are medians (IQR) for $n = 36$ in CONTROL and DAIRY, as dietary data from the FFQ were missing for 2 subjects and excluded for 2 subjects because of non-plausible energy intakes (<500 or >3500 kcal in women and <800 or >4200 kcal in men). MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TFA, trans fatty acid.

Table 6.3 Effect of DAIRY consumption on circulating lipid concentrations, surrogate markers of cholesterol homeostasis, and cholesterol trafficking measures[1]

	CONTROL	DAIRY	*P*
Serum lipids (mmol/L)			
TC	5.09 ± 0.31	5.10 ± 0.31	0.856
HDL-C	1.42 ± 0.08	1.39 ± 0.08	0.393
LDL-C	3.07 ± 0.32	3.07 ± 0.32	0.989
TG	1.30 ± 0.18	1.38 ± 0.18	0.267
TC/HDL-C ratio	3.86 ± 0.44	3.93 ± 0.44	0.211
LDL-C/HDL-C ratio	2.37 ± 0.33	2.41 ± 0.33	0.388
Plasma surrogate markers of cholesterol homeostasis			
Phytosterols (10^2 μmol/mmol TC)	371.01 ± 55.45	319.89 ± 55.45	0.0001
Campesterol	165.48 ± 25.02	152.72 ± 25.02	0.004
β-sitosterol	205.53 ± 31.05	167.17 ± 31.05	0.0001
Lathosterol (10^2 μmol/mmol TC)	146.25 ± 17.30	147.32 ± 17.30	0.82
Cholesterol clearance			
PCSK9 (ng/ml)	195.58 ± 11.73	190.91 ± 11.73	0.67
Cholesterol kinetics as measured from free cholesterol from RBCs			
Cholesterol absorption			
$AUC_{24\text{-}96\ hr}$ (‰)	857.51 ± 43.89	872.89 ± 44.24	0.66
Cholesterol synthesis			
FSR (pool/day)	0.07 ± 0.01	0.06 ± 0.01	0.43
FSR (%/day)	6.64 ± 0.59	6.36 ± 0.59	0.43

[1] Values are least-square means (LS Means) ± SEMs; n = 40. $AUC_{24\text{-}96\ hr}$, area under the [3, 4]^{13}C enrichment of RBCs cholesterol during a 24-96 hr period following the oral dose of ^{13}C-cholesterol; FSR, fractional synthesis rate; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; PCSK9, proprotein convertase subtilisin/kexin-9; TC, total cholesterol; TG, triglycerides.

6.4.3 Cholesterol absorption and biosynthesis rates

Cholesterol absorption, as measured by AUC of the ^{13}C-cholesterol RBC enrichment (872.9 ± 44.2 *vs.* 857.5 ± 43.9 ‰, $P = 0.65$), and FSR measured by deuterium incorporation (6.36 ± 0.59 *vs.* 6.64 ± 0.59 %/day, $P = 0.43$), were not modulated in response to DAIRY versus CONTROL (**Table 6.3**). Cholesterol ^{13}C enrichments at different time points also showed no significant differences between the two dietary treatments (**Figure 6.2**). In both treatments combined, area under the ^{13}C-cholesterol curve was positively correlated with plasma campesterol ($r = 0.65$), β-sitosterol ($r = 0.60$), and phytosterols ($r = 0.63$) concentrations ($P < 0.0001$ for all), and negatively correlated with plasma lathosterol concentrations ($r = -0.48$, $P < 0.0001$). On the other hand, the FSR of cholesterol (%/day) was negatively correlated with the surrogate markers of cholesterol absorption (campesterol ($r = -0.61$); β-sitosterol ($r = -0.61$); and phytosterols ($r = -0.62$), $P < 0.0001$ for all), and positively correlated with the surrogate marker of cholesterol synthesis lathosterol ($r = 0.66$, $P < 0.0001$). Correlations by the CONTROL and DAIRY treatments separately are presented in **Table 6.4**. As well, AUC of the ^{13}C-cholesterol was negatively correlated with the FSR ($r = -0.23$, $P = 0.0454$) in both treatments combined (**Figure 6.3**), indicating a not unexpected inverse relationship between cholesterol absorption and biosynthesis rates.

6.4.4 Gene-diet interactions

The responsiveness of cholesterol trafficking measures AUC and FSR to DAIRY intake, when expressed as absolute changes from CONTROL, was mediated by interactions with genetic variants in the bile acid synthesis gene *CYP7A1* SNP rs3808607 and the

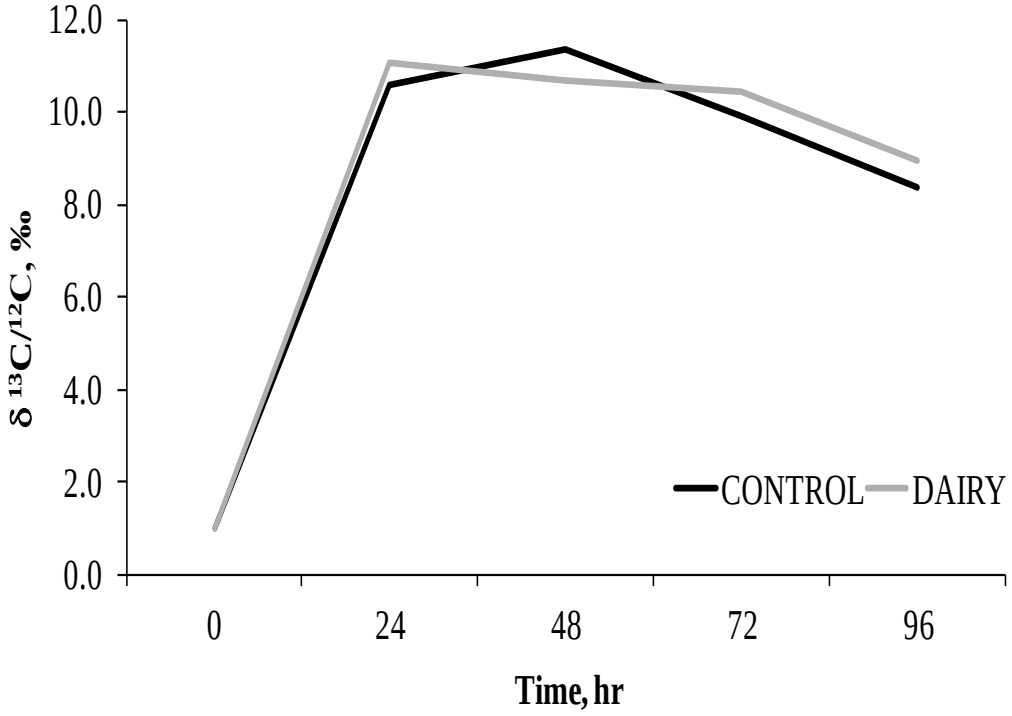

Figure 6.2 The ^{13}C enrichment of red blood cells at 24, 48, 72, and 96 hrs following the [3, 4]^{13}C-cholesterol administration in response to DAIRY and CONTROL. Values are least-square means (LS Means) ± SEMs; *n* = 40. No significant difference was observed between diets.

Table 6.4 Spearman correlation coefficients (*r*) between the surrogate markers of cholesterol homeostasis and cholesterol trafficking measures[1]

	AUC (‰)		FSR (%/day)	
	r	***P***	***r***	***P***
CONTROL				
Phytosterols (10^2 μmol/mmol TC)	0.66	<0.0001	−0.62	<0.0001
Campesterol	0.66	<0.0001	−0.57	<0.0001
β-sitosterol	0.64	<0.0001	−0.62	<0.0001
Lathosterol (10^2 μmol/mmol TC)	−0.47	0.003	0.68	<0.0001
PCSK9 (ng/ml)	−0.05	0.74	0.02	0.92
DAIRY				
Phytosterols (10^2 μmol/mmol TC)	0.63	<0.0001	−0.65	<0.0001
Campesterol	0.65	<0.0001	−0.65	<0.0001
β-sitosterol	0.57	0.0002	−0.63	<0.0001
Lathosterol (10^2 μmol/mmol TC)	−0.45	0.005	0.64	<0.0001
PCSK9 (ng/ml)	−0.02	0.90	−0.01	0.93

[1] AUC, area under the [3, 4]^{13}C cholesterol RBC enrichment curve; FSR, fractional synthesis rate; PCSK9, proprotein convertase subtilisin/kexin-9.

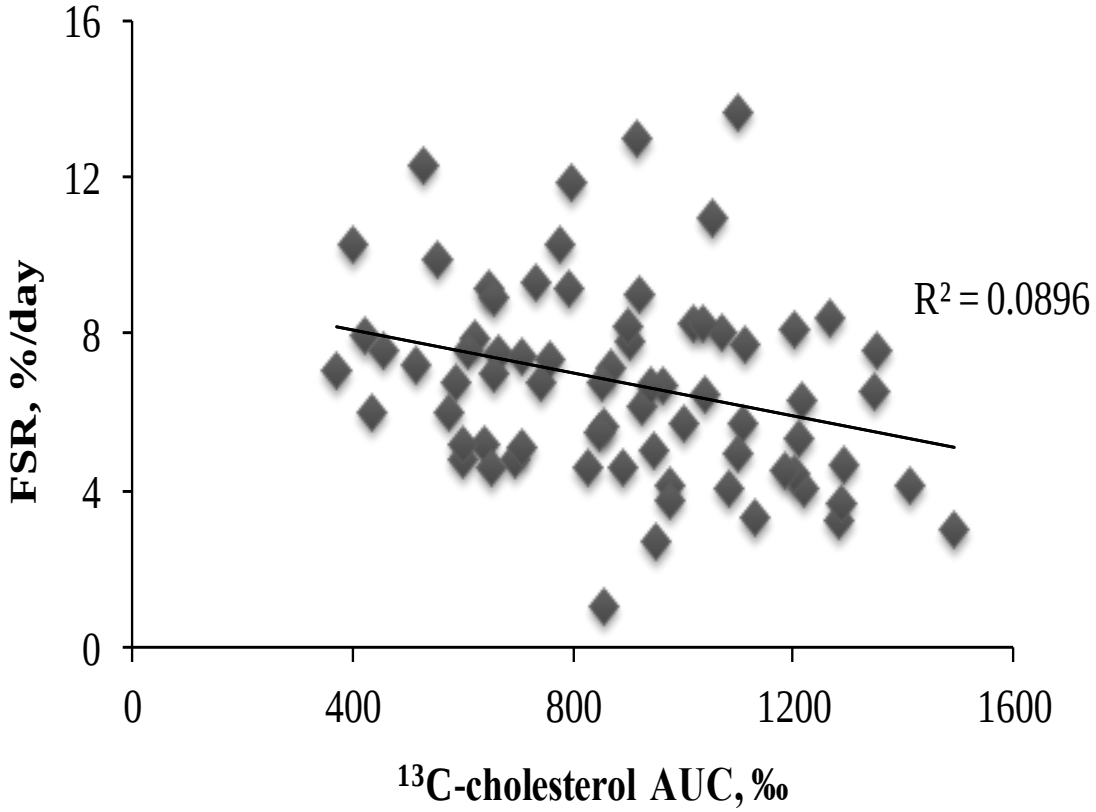

Figure 6.3 Scatter plots of association between area under the ^{13}C-cholesterol curve (AUC) and cholesterol fractional synthesis rate (FSR) (Pearson correlation coefficient data).

cholesterol synthesis gene *DHCR7* SNP rs760241 (**Table 6.5**). Specifically, compared to carriers of the *CYP7A1* rs3808607-G>T genotype, the major allele (T) homozygotes showed lower AUC (+89.5 ± 55.4 *vs.* −325.3 ± 145.5 *‰, P* = 0.0400) but not FSR (−0.47 ± 0.57 *vs.* +0.29 ± 1.06 %/day, *P* = 0.56) after DAIRY than after CONTROL. Compared to carriers of the *DHCR7* rs760241-G/G genotype, however, carriers of the minor allele (A) showed lower FSR (+0.43 ± 0.45 *vs.* −1.62 ± 0.78 %/day, *P* = 0.0336) but a trend of higher AUC (−32.6 ± 54.5 *vs.* +170.9 ± 88.5 *‰, P* = 0.07).

6.5 DISCUSSION

The main findings of the present study were that, firstly, short-term recommended level of conventional dairy product intake in the context of a prudent diet, compared with non-dairy products, does not modify the overall cholesterol absorption or synthesis in healthy subjects. Secondly, the study confirms an established reciprocal relationship between cholesterol absorption and synthesis rates. And finally, carriers of certain variants of key cholesterol-related genes show differing absorption and synthesis rates after consumption of the dairy diet.

The association between dairy product intake and cholesterol concentrations in blood, or cardiovascular health, has long been examined. Akin to some (38-41), but not all previous research (42-45), we observed no effect of dairy consumption on circulating cholesterol concentrations. Our findings also complement those of a meta-analysis by Benatar et al. (46), where no significant changes in HDL-C or LDL-C concentrations were observed after increasing dairy food consumption in nine studies that together involved 700 individuals. The mixed findings in the current literature are likely attributed

Table 6.5 Impact of genetic variants of cholesterol-related genes on the response of cholesterol trafficking measures to DAIRY[1]

	***CYP7A1* rs3808607**				***DHCR7* rs760241**		
	T/T (*n* = 4)	**G>T (*n* = 15)**	**G/G (*n* = 6)**	***P***	**G/G (*n* = 19)**	**A>G + A/A (*n* = 6)**	***P***
Δ AUC (*‰)*	−325.3 ± 145.5[a]	89.5 ± 55.4[b]	−12.7 ± 84.0[ab]	0.0400	−32.6 ± 54.5	170.9 ± 88.5	0.07
Δ FSR (%/day)	0.3 ± 1.1	−0.5 ± 0.6	0.6 ± 0.9	0.56	0.4 ± 0.5	−1.6 ± 0.8	0.0336

[1] Values are least-square means (LS Means) ± SEMs of absolute changes from endpoint CONTROL in response to endpoint DAIRY; *n* = 25. Values within a row with different superscript letters are significantly different (ANCOVA procedure with Tukey adjustments for sex). AUC, area under the [3, 4]^{13}C cholesterol RBC enrichment curve; *CYP7A1*, cholesterol 7-alpha-hydroxylase; *DHCR7*, 7-dehydrocholesterol reductase; FSR, fractional synthesis rate.

to differences in the nature of dairy products employed in various studies (47) or other research-related factors (19, 20). For instance, of all dairy products, butter consumption has shown the least controversial impact on cholesterol concentrations (47-49), whereas data on milk, yogurt, and cheese intakes are less conclusive but mostly indicative of protective effects on both cholesterol concentrations (17, 19, 50, 51) and cardiovascular health (20, 23, 52-57).

Plasma concentrations of phytosterols, in particular campesterol and β-sitosterol, have been shown to serve as valid surrogate non-cholesterol sterol markers of intestinal cholesterol absorption (32), and lathosterol as an endogenous cholesterol synthesis marker (32). As such, the lower concentrations of plasma phytosterols after DAIRY observed in the present work may be suggestive of a reduced intestinal absorption of sterols with intakes of dairy products, and agree with our previous observations of an association between high *trans* fat intake from ruminants sources and decreased cholesterol absorption (58). However, given the nature of the study's food products, it is perhaps also likely that this observation is attributed to higher dietary cholesterol intake during DAIRY, higher phytosterol intake in CONTROL, or a combination of both effects; even though plant sterols in food are poorly absorbed (10).

It is known that at the intestinal level, plant sterol and cholesterol molecules compete for incorporation into the mixed micelles (59, 60) and that higher intakes of plant sterols lower cholesterol absorption and, as a result, its concentrations in blood (9). On average, the combined dairy products of the current trial had 50 mg higher cholesterol content than the control products (27) and there was an approximately 45 mg/day difference in the

self-reported daily intakes of dietary cholesterol when participants were on DAIRY versus when they were on CONTROL (**Table 6.2**). As well, the CONTROL phase of this trial contained products that are typically higher in plant sterols, mainly cashews, a 20 g of which contains ~30 mg of plant sterols (61). Trafficking measures of cholesterol absorption (as assessed by the stable isotopes) further showed no between-treatment differences, supporting calls for caution in interpreting data of cholesterol absorption surrogates in situations where plant sterols are supplemented (62).

Regardless of the lack of changes in the ^{13}C-cholesterol enrichment and FSR in this trial, our findings of positive correlations between these kinetics measures and the surrogate markers of cholesterol absorption and synthesis, respectively, support those of previous research (11). Also, we here confirm an established reciprocal interplay between cholesterol absorption and synthesis rates (63, 64) in the context of dairy and dairy-free diets. Given the importance of cholesterol in the structure and function of cell membrane and in serving as a substrate for the biosynthesis of steroid hormones, the balance of cholesterol absorption and synthesis is critical. This is also evident in clinical practices where the use of inhibitors of cholesterol absorption or synthesis has been an effective strategy for regulating cholesterol concentrations in blood (64).

PSCK9 has been identified in the regulation of cholesterol homeostasis (65). In mice lacking the *PCSK9* gene, reductions in plasma TC concentrations were evident and attributed to an increased activity of LDL receptors (66). Also, stable isotope studies in humans have revealed an inverse correlation between plasma PCSK9 and with LDL clearance (12). Similar to a previous crossover placebo controlled study where we tested

impacts of buttermilk consumption on plasma lipids and surrogate markers of cholesterol homeostasis (67), we observed no significant change in plasma PCSK9 concentrations after DAIRY in this trial. Together, these findings suggest no effect of dairy products consumption on this novel marker of LDL clearance.

We have recently shown a genetic basis for circulating TC and LDL-C responsiveness to DAIRY among a larger group of individuals in this trial (under review). Here we further provide first evidence of a gene-diet interaction affecting the cholesterol trafficking as determined by the stable isotope tracers, an established method of cholesterol absorption and biosynthesis (9, 68). Whereas no studies have thus far assessed the influence of SNPs within genes encoding metabolites of cholesterol homeostasis on responses of cholesterol kinetics to dairy consumption, circulating cholesterol concentrations have in particular been examined with diets high in SFA (69) and dietary cholesterol (70). For instance, in agreement with other studies (71), Hofman et al. (72) reported 60% lower plasma TC concentrations among the T/T homozygotes of the *CYP7A1* SNP rs3808607 (MAF = 0.4496) with higher intakes of dietary cholesterol than among the G allele carriers. *CYP7A1* gene encodes cholesterol 7 alpha-hydroxylase, which is the rate-limiting enzyme in bile acid biosynthesis. On the other hand, the *DHCR7* gene encodes the 7-dehydrocholesterol reductase, which catalyzes the conversion of 7-dehydrocholesterol to cholesterol. Certainly, these genes play important roles in cholesterol metabolism, but it is hard to draw a firm conclusion as to the exact mechanisms of action or clinical implications of their interactions with the dairy diet of this trial. Unlike the majority of gene-diet interaction studies in the literature, which test single nutrients, dairy is a food group that constitutes many products, and nutrients, that may individually show different

effects in interaction with some but not other genotypes of interest. Another challenge in interpretation relates to the possible synergistic interactions with unidentified lifestyle factors or other genes involved in cholesterol regulation. In any case, the lower cholesterol absorption rate observed among the *CYP7A1* rs3808607-T/T homozygotes and lower cholesterol biosynthesis rate among the *DHCR7* rs760241-A allele carriers are novel observations that warrant replication through carefully controlled feeding studies for better understanding of the clinical significance.

To our knowledge, this study is the first to examine the cholesterol homeostasis through surrogate markers of cholesterol absorption and synthesis, together with stable isotope tracer (cholesterol kinetics) techniques in the context of a recommended level of dairy consumption. The research also provided detailed phenotypic and metabolic evaluations of gene-diet responses according to several polymorphisms of established roles in cholesterol metabolism. The crossover design and use of commercially-available dairy products that are usually consumed by the general public, in a context of a healthy dietary pattern, represent other strengths. However, limitations such as the unevenly distributed frequencies of genetic variants in the genes under study and the relatively small sample size for such genetic analyses cannot be neglected.

In summary, adherence to a healthy-style dietary pattern with 3 servings/day of conventional low-fat and regular dairy products for 4 weeks appears to have limited impact on overall cholesterol homeostasis. However, carriers of certain genetic variants in cholesterol-related genes may show lower cholesterol absorption or biosynthesis rates in response to a dairy diet. Prospective genotyping studies with larger sample sizes will

ensure better views into the clinical implications of these findings on the long run in a way that could allow identification of individuals who could benefit from dairy intake versus those who could not.

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The authors' responsibilities were as follows: MMHA coordinated the study, performed the laboratory and statistical analyses, interpreted the data, and wrote the manuscript. PKE contributed expertise for the genotyping studies. BL, PC, and PJ designed and supervised the study. PC was responsible for the screening and medical supervision of the

study participants. BL and PJ had primary responsibility for the final content. All authors critically reviewed the manuscript and approved its final version.

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BRIDGE TO CHAPTER VII

Many factors can shape the cardiovascular health, both in etiology and treatment. The effect of diet on multiple rather than individual biomarkers of CVD is thus logically better in assessing potential risk. The inflammatory state is a more recent biomarker of CVD than elevated cholesterol concentrations, but similar to data on cholesterol the impact of dairy intake on inflammatory markers is still somewhat debatable. Chapter VII presents findings of the dairy intake in the present work in relation to systemic inflammation and expression of inflammatory genes, the secondary objective of research in this thesis. Mohammad Abdullah was the study coordinator at the Winnipeg site where he was responsible of data collection and all other research aspects, and critically reviewed the manuscript in the next chapter for important intellectual content.

CHAPTER VII

MANUSCRIPT 5

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DAIRY PRODUCT CONSUMPTION HAS NO IMPACT ON BIOMARKERS OF INFLAMMATION AMONG MEN AND WOMEN WITH LOW-GRADE SYSTEMIC INFLAMMATION

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7.1 ABSTRACT

Background: Randomized controlled trials specifically designed to assess inflammation-related outcomes in response to dairy consumption are lacking. **Objective:** We investigated the impact of dairy food consumption on biomarkers of inflammation in healthy men and women with low-grade systemic inflammation. **Methods:** In a multicentre randomized crossover study, 112 adult men and women with high-sensitivity C-reactive protein (*hs*-CRP) values > 1 mg/L consumed 3 servings/day of DAIRY (375 mL low-fat milk, 175 g low-fat yogurt and 30 g regular fat cheddar cheese) or energy-matched CONTROL products (fruit juice, vegetable juice, cashews and one cookie) as part of prudent 4-week diets, each separated by a 4-8 week washout period. Serum concentrations of inflammation biomarkers were measured at the beginning and end of each dietary phase. Expression levels of key inflammatory genes and transcription factors in whole blood cells were assessed at the end of each diet by real-time polymerase chain reaction in a random subset of 53 subjects. **Results:** Analysis of within-diet changes (post- *vs.* pre-diet values) showed a significant reduction in *hs*-CRP concentrations after CONTROL (−11.7%, $P = 0.05$), but no change after DAIRY (−7.3%, $P = 0.47$). As a result, changes in *hs*-CRP differed between DAIRY and CONTROL ($P=0.04$). Both the CONTROL and DAIRY diets similarly reduced interleukin (IL)-6 concentrations compared with diet-specific baseline values (−17.6% and −19.9%, respectively, $P < 0.0001$ for both, $P = 0.77$ for between-diet comparison). No between- nor within-diet difference was observed in adiponectin concentrations and there was also no between-diet difference in the expression of inflammatory genes and transcription factors. **Conclusions:** Consistent with data from previous work, these results suggest that short-

term consumption of a combination of low- and high-fat dairy products as part of a healthy diet has no adverse effects on inflammation.

7.2 INTRODUCTION

A growing body of evidence from epidemiological studies suggests that milk and dairy product consumption is associated with a modest but significant reduction in the risk of cardiovascular disease (CVD) (1), coronary heart disease (2-4) and type 2 diabetes (2-8). Dairy product consumption may also be protective against metabolic syndrome, although this evidence remains equivocal (9). The presence of an underlying pro-inflammatory state, characterized by increased circulating concentrations of C-reactive protein (CRP) and inflammatory cytokines (e.g. IL-6 and TNF-α) as well as decreased concentrations of anti-inflammatory mediators such as adiponectin, plays a central role in the pathophysiology of several disorders, including metabolic syndrome and CVD (10-12).

Conflicting results emerge regarding the impact of dairy products on inflammation. Earlier cell culture studies have shown that incubating mononuclear cells with lactic acid bacteria stimulates the production of pro-inflammatory cytokines (13-15). Yet in humans, cross-sectional studies have quite consistently showed that consumption of dairy, particularly low-fat dairy products, as part of a healthy diet was associated with less systemic inflammation (16-19). We have recently shown in a systematic review of randomized controlled nutritional intervention studies that dairy product consumption did not promote a pro-inflammatory state in overweight and obese adults (20). However, definite conclusions on a beneficial impact of dairy products on inflammation could not be drawn. Our systematic review stressed the need for additional studies on this topic, with particular emphasis on considering inflammation as a primary study outcome.

The primary objective of this randomized nutritional intervention study was to investigate the impact of dairy food consumption on biomarkers of inflammation in healthy men and women with low-grade systemic inflammation. Based on the apparent cardioprotective effects of dairy products, we hypothesized that dairy food consumption has favorable effects on the inflammatory profile. We also investigated how dairy food consumption modifies the expression of inflammatory genes in whole blood cells.

7.3 MATERIALS AND METHODS

7.3.1 Participants

This research was undertaken as a multicentre study involving two academic Canadian research centres, the Institute of Nutrition and Functional Foods (INAF) in Quebec City and the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN) in Winnipeg. Men and women were recruited between January and September 2011 through the media and electronic newsletter to participate in the study. For eligibility, participants had to be between 18-70 y of age with sub-clinical inflammation as assessed by two consecutive measurements of plasma high-sensitivity (*hs*)-CRP concentrations above 1.0 and below 10.0 mg/L. The *a priori* defined lower limit of 2.0 mg/L for *hs*-CRP was changed to 1.0 mg/L during the course of the study to facilitate recruitment. There was no eligibility criterion based on usual dairy intake but participants were asked to reduce their dairy intake to 2 servings/day or less during the run-in period. Exclusion criteria included: smoking, body weight variation over the last 6 mo greater than 10%; a BMI over 35 kg/m^2; a previous history of CVD, type 2 diabetes, monogenic dyslipidemia, or any diagnosed endocrine or gastrointestinal disorder; the use of anti-hypertensive, anti-

inflammatory or lipid-lowering medication (i.e. statins); allergies and intolerance to dairy foods; aversion to foods provided during the study and clinical use of vitamin D and calcium supplements. The *a priori* defined cutoff of 35 kg/m^2 for BMI was modified during the recruitment phase to include two participants (one in each centre) with a BMI of 36.3 kg/m^2. Among premenopausal women, only those with a regular menstrual cycle (25–35 d) for the past 3 mo were included. All postmenopausal women were eligible irrespective of their hormone supplementation status as long as it remained constant throughout the study. Follicle-stimulating hormone (FSH) measurements were performed to confirm the premenopausal or postmenopausal status (FSH < 20 IU/L or FSH > 25 IU/L). Women who had initiated hormone replacement therapy within 6 mo of study onset were not eligible. Women who used contraceptive agents were not excluded from the study. Participants were enrolled by MCL and AC at INAF and by MMA at RCFFN. The study protocol was fully explained to all participants, who gave written informed consent at the time of enrollment. The study protocol was in accordance with the ethical standards of each institution involved. A total of 212 men and women were found to be eligible (**Figure 7.1**), among whom 137 were randomized to one of the two intervention sequences. Seven participants never started the intervention and six dropped out due to the incapacity to commit to the demanding nature of the protocol. Of the remaining 124 participants who completed the two dietary phases, twelve were excluded from analyses because of a post-diet value of *hs*-CRP above 10 mg/L in at least one of the two dietary phases, which is an indication of an acute inflammatory response (21). Retaining these participants in the analyses did not materially alter the results (not shown).

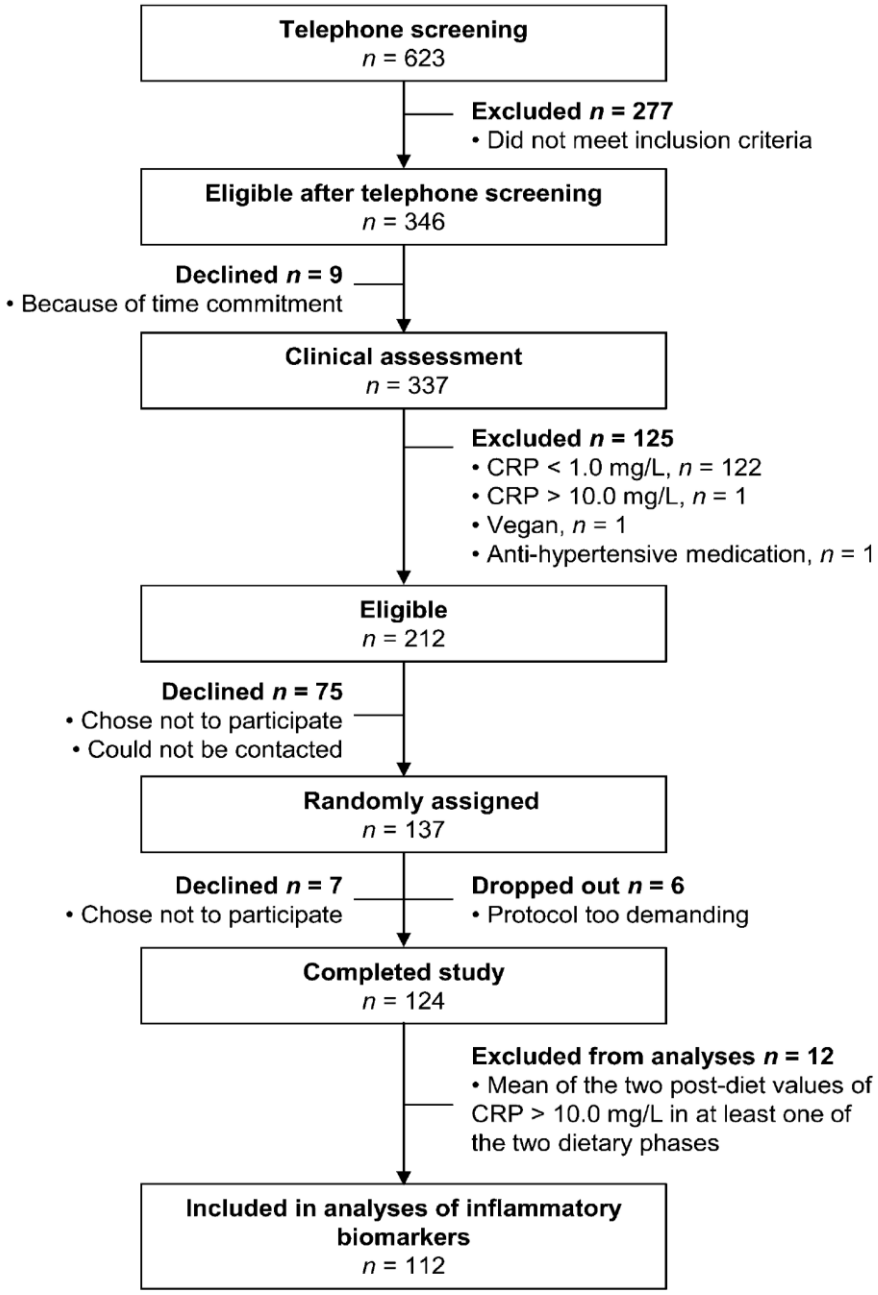

Figure 7.1 Flow of participating men and women with low grade inflammation throughout the study

7.3.2 Study design and diets composition

The study was undertaken using a randomized, crossover design with two 4-week treatments, DAIRY and CONTROL. During a 2-week run-in period, enrolled participants received dietary advice by a registered dietitian on how to adapt their diet to a prudent dietary pattern characterized by low intakes of SFA (< 10% energy), *trans* fat (< 1% energy), dietary cholesterol (< 200 mg/day) and sodium (< 2300 mg/day) (22). The total amount of fat was not restricted, with recommendations ranging from 25-35% of total energy. Substituting saturated by unsaturated fat was advocated. Advice on how to restrict the consumption of processed and energy-dense foods were provided, with also a specific recommendation to consume a maximum of 2 servings of dairy products per d during the run-in. No food was provided to participants during this 2-week run-in period. Randomization of participants to one of the two diet sequences (DAIRY/CONTROL or CONTROL/DAIRY) was computer-generated after the run-in period by a member of the research team at INAF without any knowledge of participants' status and eligibility. The sequence for each participant was thereafter passed on to the study coordinators at both centres. Randomization to diet sequences within each centre was further stratified for baseline *hs*-CRP concentrations (1-2 mg/L and 2-10 mg/L based on the screening results) and sex. The backbone of the CONTROL and DAIRY diets corresponded to the prudent dietary pattern recommended during the run-in period. However, during the DAIRY diet, participants had to specifically incorporate 3 servings of dairy products into their everyday diet. The following combination of low- and high-fat dairy products was provided to participants on a weekly basis by the research team: 375 mL/day of low-fat milk (1% M.F.), 175 g/day of low-fat yogurt (1.5% M.F.) and 30 g/day of regular fat

cheddar cheese (34% M.F.). During the CONTROL diet, participants were provided with the following energy-equivalent control products on a weekly basis: 290 mL/day of 100% fruit juice (Oasis® Classic line), 156 mL/day of vegetable juice (V8® Original Vegetable Cocktail) (23), 20 g/day of cashews and one cookie (39 g/day) prepared in the metabolic kitchen of each research centre using a standardized recipe. Available fruit juice flavours were orange, apple or wildberry. Orange and apple were the most popular flavours among participants. The nutritional composition of all three flavours was similar (24). Consumption of dairy products during the CONTROL diet was forbidden. The composition of DAIRY and CONTROL foods is presented in **Table 7.1**. The two diets were separated by a 4- to 8-week washout period during which participants were also asked to comply with the prudent dietary pattern described above. Participants were asked to maintain their body weight and usual levels of physical activity constant throughout the duration of the study. They were specifically told that this was not a weight loss study. A 3-d physical activity journal was collected at the end of each dietary phase. Consumption of tea and coffee (without milk or cream) was allowed within a limit of 2 cups (500 mL)/day. Alcohol was restricted to 1 drink/day, up to 5 per week (1 drink was considered as a normal size bottle of beer [340 ml], a glass of wine [150 ml] or a glass of liquor [45 ml]). Supplementation with vitamins and natural health products was strictly forbidden throughout the study. Anti-inflammatory medication such as ibuprofen was also prohibited during the intervention. Study coordinators and participants were not blinded due to the nature of the interventions.

Table 7.1 Average nutritional composition of foods provided as part of the CONTROL and DAIRY diets

Nutrient	**CONTROL**	**DAIRY**
	Fruit juice (290 ml) Vegetable juice (156 ml) Cashews (20 g) Cookie (39 g)	Low fat milk (375 ml) Yogurt (175 g) Cheese (30 g)
Energy (kJ)	1833	1807
Total fat (g)	17.1	16.3
SFA	7.6	8.7
MUFA	6.6	4.1
PUFA	1.91	0.42
TFA[1]	0.01	0.08
Protein (g)	7.7	26.0
Carbohydrate (g)	63.3	45.0
Fibre (g)	3.2	0
Sodium (mg)	459	465
Calcium (mg)	84	913
Vitamin D (μg)	0	5.2

[1] TFA, *trans* fatty acids.

7.3.3 Dietary assessment

Dietary intake was assessed at screening and at the end of each dietary phase using a validated and highly reproducible web-based self-administered FFQ (25), which justifies its use in nutrition intervention studies assessing dietary intakes of participants over time, as is the case in the present crossover study. Intakes of specific food groups in terms of servings/day obtained from the web-FFQ were calculated based on servings of Canada's Food Guide (26).

7.3.4 Compliance

Compliance to the dietary recommendations was assessed using data from the web-FFQ and a checklist provided each week to identify DAIRY or CONTROL foods that were consumed or not. This list also provided space to indicate alcohol, tea and coffee consumption as well as medication use. Participants had to notify the physician in charge of the clinical aspects of the study before initiating any medication. Behavioural and psychological counseling was offered to participants to maximize the success of the nutritional changes. Compliance was also assessed by measuring serum 25-hydroxyvitamin D (25(OH)D) concentrations after the DAIRY and CONTROL dietary phases. The concentration of 25(OH)D is expected to increase when dietary intake of vitamin D is increased (27).

7.3.5 Clinical measures at screening

Systolic and diastolic blood pressures at screening were averaged from 3 measurements taken after a 10-min rest in the sitting position with an automated blood pressure monitor

(BPM 300-BpTRU model; Vital Signs Monitor). Serum lipid profile at screening was performed according to methods described previously (28-31). *hs*-CRP concentrations at screening were measured by nephelometry as previously described (32). The CV of this assay is <1% for both low and high *hs*-CRP concentrations (32).

7.3.6 Anthropometric measures

Body weight, waist and hip circumferences were measured according to standardized procedures at screening as well as at the beginning and at the end of each dietary phase (33). Body weight was specifically measured twice within the last week of each diet. The mean of the two post-diet values of body weight was used for the calculation of BMI post-diet. Lean and fat mass were measured by the DXA technique (GE Healthcare, Madison, WI) once at the end of each dietary phase.

7.3.7 Inflammatory biomarkers

Fasting blood samples (12-hr) were collected from an antecubital vein once at the beginning and twice within the last week of each dietary phase, 2-5 day apart. The mean of the two post-diet values was used in analyses of *hs*-CRP, IL-6 and adiponectin in order to minimize intra-individual variability. Serum *hs*-CRP concentrations were measured using a commercial ELISA kit for the human form (BioCheck Inc, Foster City, CA). Serum IL-6 (R&D Systems Europe, Inc.) and adiponectin concentrations (B-Bridge International, Inc.) were also measured using commercial ELISA kits. The intra-assay CV were 4.4%, 7.4% and 5.2% for *hs*-CRP, IL-6 and adiponectin, respectively. The laboratory staff was blinded to the dietary interventions.

7.3.8 Inflammatory gene expression analysis

Samples were collected using PAXGene Blood RNA tubes (Qiagen, Valencia, CA) at the end of each dietary phase for the direct measurement of inflammatory gene expression in whole blood cells in a random subsample of 53 participants. Total RNA was first isolated from 2.5 mL of whole blood samples using the PAXGene Blood RNA purification kit (Qiagen, Valencia, CA) according to manufacturer's instructions. RNA was thereafter quantified using NanoDrop (Thermo Fisher, Wilmington, DE). cDNA was synthesized from 1 μg of RNA using the High Capacity cDNA Reverse Transcription Kit (Life Technologies). Gene expression was measured by qRT-PCR using TaqMan® OpenArray® Real-Time PCR Plates with Inventoried Gene Expression Assays (Applied Biosystems, Foster City, CA) and the QuantStudio 12K Flex Software (Applied Biosystems, Foster City, CA). GenBank and Life Technologies numbers of the analyzed genes are provided in **Supplemental Table 7.1**. Each sample was analyzed in triplicate. Expression of the target genes was normalized to the expression of the internal control gene glucose-6-phosphate dehydrogenase (*G6PD*) using the formula ΔCt = (Ct value of the target gene – Ct value of the internal control gene) (34). Ct values used in the formula consisted of the mean of the triplicates' individual Ct values, unless the SD of a triplicate was > 0.5, in which case the outlier value was excluded from the calculation of the mean. Expression of the following 10 inflammation-related genes and transcription factors was measured: chemokine (C-C motif) ligand 2 (*CCL2*), *IL18*, *IL6*, *IL1B*, nuclear factor kappa-B subunit 1 (*NFKB1*), natriuretic peptide receptor C (*NPR3*), peroxisome proliferator-activated receptor alpha (*PPARA*), sterol regulatory element-binding transcription factor 2 (*SREBF2*), *TNF*, and TNF receptor-associated factor 3 (*TRAF3*).

CCL2, *IL6* and *NPR3* were not retained in the analyses since their level of expression was too low to be detected in approximately half of the samples and thus did not allow reliable data to be obtained. Normalization of the target genes to the expression of the internal control gene *GAPDH* was also considered and led to similar results, but *G6PD* showed the smallest variation in expression after the DAIRY *vs.* CONTROL diet (fold change −1.01 for *G6PD vs.* 1.05 for *GAPDH*).

7.3.9 Sample size calculation

Sample size estimates were calculated based on data from Zemel *et al.* (35). When combining the various groups in that study (overweight, obese, all) and the 2 time points during the intervention (7 and 28 day), the SD of the change in plasma *hs*-CRP in response to dairy was on average 300% (3-fold) greater than the actual change in mg/L in that study (35). Our anticipated dropout rate was set at 15%. Based on these considerations and on the assumption that the average *hs*-CRP concentration at baseline would be around 3 mg/L as per our inclusion criteria, we have calculated that the study with 120 subjects completing the two phases of the intervention would allow us to detect a clinically meaningful 0.8 mg/L (25%) difference in *hs*-CRP concentrations between the DAIRY and CONTROL diets, with a power of 82% and a type 1 error (α) of 5%. Between-diet differences in other markers of inflammation, anthropometry indices and relative expression levels of inflammatory genes represented secondary study outcomes.

7.3.10 Statistical analyses

The pre-specified primary analysis was undertaken using a *per protocol* approach. This analysis consisted of assessing within-diet changes in study outcomes (i.e. delta scores calculated as post-diet [week 4] minus pre-diet [week 0] values in the DAIRY and CONTROL diets) as well as between-diet (DAIRY *vs.* CONTROL) differences in delta scores using the MIXED procedure for repeated measures in SAS (version 9.2; SAS Institute, Cary, NC), with diet as a fixed effect and subject as a random effect. The significance of within-diet changes was determined using the least-square means statistic in the MIXED procedure. The pre-specified secondary analysis consisted of comparing post-diet values of outcome variables between the DAIRY and CONTROL diets also using the MIXED procedure, with diet as a fixed effect and subject as a random effect. The structure of the covariance matrix for each variable was taken into account in all analyses to ensure the most adequate statistical fit of the model to the experimental data. Pre-specified potential confounders of the inflammatory markers' response to diet, namely sex, study centre, and pre-diet inflammatory status, were included as covariates if they were found to be significant at $P < 0.05$ in a selected model. Further adjustment for anthropometry indices (i.e. waist circumference or BMI) had no impact on the results and thus was not retained in any of the analyses. The interaction of the above mentioned potential confounders as well as the interaction of age and menopausal status with the main treatment effect was also tested in all analyses using appropriate interaction terms and multivariate modeling. No such interactions were found. Analyses also showed no evidence of a carry-over effect of the dietary treatments on inflammatory outcomes (not shown), except for the relative *IL1B* gene expression levels (described in the Results

section). Univariate correlations among key outcomes were assessed using Spearman correlation coefficients. Baseline characteristics of the random subsample of 53 subjects used for gene expression analyses were compared with those of the whole study sample using the Student's unpaired t-test. Variables with a normal distribution are reported in the text as means ± SD. Abnormally distributed variables were natural log-transformed before statistical analysis and are reported in the text as geometric means (95% CI), except data pertaining to dietary intakes. Indeed, dietary intakes were analyzed using non-parametric tests (Friedman test or Wilcoxon matched-pairs signed-rank test) and data are therefore reported as medians (IQR). Frequencies are reported in the text as % with number of participants in parentheses. Differences at $P \leq 0.05$ were considered significant.

7.4 RESULTS

The 112 subjects who were included in the final *per protocol* analysis had a mean (± SD) age of 40.1 ± 16.7 yr and were primarily women (66.1%, *n* = 74) and Caucasian (66.7%, *n* = 74) (**Table 7.2**). They were in good health with average blood pressure and cholesterol concentrations within acceptable ranges (36). The mean concentration of *hs*-CRP at screening was 2.51 mg/L (geometric mean, 95% CI 2.29-2.74). Among women, 35.1% (*n* = 26) were postmenopausal and 6.8% (*n* = 5) had been using hormone replacement therapy for more than 6 mo. Among premenopausal women, 43.8% (*n* = 21) were using oral contraceptives. Participants' intake of dairy products was 2.0 servings/day (median, IQR: 1.9, **Table 7.3**) at screening. We calculated based on checklists that 98.5 ± 2.8% and 96.6 ± 5.9% (means ± SD) of the DAIRY and

Table 7.2 Characteristics of the 112 men and women with low grade inflammation at screening[1]

	Mean ± SD[2]	**(min-max)**
Women [n (%)]	74 (66)	
Postmenopausal women [n (% of women)]	26 (35)	
Ethnicity[3] *[n (%)]*		
Caucasian	74 (67)	
Asian	28 (25)	
African	3 (3)	
Hispanic	3 (3)	
Other	3 (3)	
Age (y)	40.1 ± 16.7	(18-69)
Body weight (kg)	72.2 ± 15.6	(46.3-122.4)
BMI (kg/m^2)	25.8 ± 4.3	(17.4-36.3)
Waist circumference (cm)	87.5 ± 13.6	(61.5-118.0)
hs-CRP[4] (mg/L)	2.51 [2.29-2.74]	(1.06-8.47)
Blood pressure[5] *(mm Hg)*		
Systolic	114.2 ± 12.4	(94.7-148.0)
Diastolic	67.6 ± 7.3	(53.3-84.0)
Plasma lipids[6] *(mmol/L)*		
TC	5.3 ± 1.0	(3.1-7.9)
LDL-C	3.0 ± 1.0	(1.2-5.3)
HDL-C	1.7 ± 0.4	(0.9-2.8)
TG	1.4 ± 0.6	(0.6-3.2)
TC/HDL-C ratio[6]	3.3 ± 1.0	(1.8-7.0)

[1] HDL-C, HDL-cholesterol; *hs*-CRP, high-sensitivity C-reactive protein; LDL-C, LDL-cholesterol; TC, total cholesterol. [2] Values are means ± SD unless indicated otherwise, i.e. frequency (%) or geometric mean [95% CI]. [3] Percent values based on $n = 111$ as data on ethnicity was missing for one participant. [4] Value is the geometric mean [95% CI].

[5] Values at screening available for only 52 participants because they were not measured in one of the two centres. [6] Values at screening available for only 51 participants because they were not measured in one of the two centres.

Table 7.3 Self-reported intakes by food groups of participants' usual diet as well as during the CONTROL and DAIRY 4-week diets in men and women with low grade inflammation[1]

Food groups	USUAL	CONTROL	DAIRY	*P*[2]
	servings/day			
Dairy products	2.0 (1.9)	0.0 (0.3)[a]	3.5 (1.0)[a,b]	<0.0001
Milk	0.6 (1.3)	0.0 (0.0)[a]	1.6 (0.5)[a,b]	<0.0001
Yogurt	0.2 (0.3)	0.0 (0.0)[a]	0.9 (0.0)[a,b]	<0.0001
Cheese	0.3 (0.9)	0.0 (0.0)[a]	0.6 (0.0)[a,b]	<0.0001
Fruits and vegetables	5.5 (3.9)	8.7 (5.1)[a]	5.5 (5.1)[b]	<0.0001
Fruit juice	0.3 (0.9)	2.0 (1.4)[a]	0.2 (1.0)[b]	<0.0001
Vegetable juice	0.0 (0.2)	1.3 (0.0)[a]	0.0 (0.2)[a,b]	<0.0001
Grain products	4.5 (3.1)	3.7 (2.8)[a]	4.1 (2.5)[a]	<0.0001
Meat and alternatives	2.2 (1.8)	2.5 (1.8)[a]	1.9 (1.8)[a,b]	<0.0001
Animal protein	1.5 (1.2)	1.5 (1.2)	1.5 (1.2)[a]	0.01
Vegetable protein	0.4 (0.6)	0.9 (0.8)[a]	0.4 (0.6)[b]	<0.0001
Nuts	0.1 (0.2)	0.6 (0.1)[a]	0.1 (0.2)[b]	<0.0001
Other foods				
Cookies	0.2 (0.4)	1.0 (0.7)[a]	0.2 (0.4)[b]	<0.0001

[1] Values are medians (IQR). n = 108 for the USUAL intakes (i.e. measured at screening) as dietary data for 4 participants were excluded because of non-plausible energy intakes (< 2092 or > 14 644 kJ [< 500 or > 3500 kcal] in women and < 3347 or > 17 573 kJ [< 800 or > 4200 kcal] in men); n =106 in the CONTROL diet as dietary data from the FFQ were missing for 3 participants and 3 participants were excluded because of non-plausible energy intakes; n = 104 in the DAIRY diet as dietary data were missing for 6 participants and 2 participants were excluded because of non-plausible energy intakes. [2] P values for differences between measurements, as determined by the Friedman test. Pairwise comparisons were performed using rank transformation of the data followed by the general linear model procedure and the Tukey adjustment for multiple comparisons. [a] = significantly different from USUAL ($P < 0.05$); [b] = significantly different from CONTROL ($P < 0.05$).

CONTROL by participants during the intervention periods. Self-reported intakes of the test foods during each phase also matched very closely the number of servings provided to participants (**Table 7.3**). Serum concentrations of 25(OH)D were significantly higher after DAIRY (76.4 ± 27.8 nmol/L) than after CONTROL (68.2 ± 29.0 nmol/L, P = 0.0004).

Intakes of grain products and animal proteins in terms of servings per d did not differ between DAIRY and CONTROL (**Table 7.3**). However, intakes of dairy products, fruits and vegetables, meat and alternatives, and vegetable proteins all differed between the two diets (all $P \leq 0.0006$). In terms of nutrient intake, participants while on DAIRY consumed more energy, protein, SFA, dietary cholesterol, calcium and vitamin D, but less carbohydrate, fibre, MUFA and PUFA compared with CONTROL (all $P < 0.05$) (**Supplemental Table 7.2**). However, after having removed food items provided to participants during the CONTROL and DAIRY diets from the self-reported dietary intake data, the remainder of these diets was very similar (**Supplemental Table 7.3**). The main residual differences in participants' background diet were a higher consumption of fruits (mostly from fruit juice), energy and carbohydrate and a lower consumption of protein in DAIRY *vs.* CONTROL (**Supplemental Table 7.3**).

There was a small increase in BMI after DAIRY (within-diet change of 0.4%, P = 0.001, **Table 7.4**), which remained significant after adjustment for energy intake. Within-diet changes in BMI as well as post-diet values of BMI also differed between CONTROL and DAIRY (P = 0.0007 for both), but again differences were very small and remained significant after adjustment for energy intake. However, differences in BMI between

Table 7.4 Effects of the CONTROL and DAIRY diets on anthropometry indices and inflammatory biomarkers in the 112 men and women with low grade inflammation[1]

	CONTROL				DAIRY				Between-diet *P*	
Variable	Pre	Post	Δ (95% CI)[2]	%	Pre	Post	Δ (95% CI)[2]	%	Δ *vs.* Δ[3]	Post *vs.* Post[4]
Anthropometry										
BMI[5] (kg/m^2)	25.8 ± 4.3	25.8 ± 4.3	-0.04 (-0.10, 0.02)	-0.1	25.8 ± 4.3	25.9 ± 4.4	0.10 (0.04, 0.16)*	0.4	0.0007	0.0007
Waist circumference[6] (cm)	86.4 ± 14.1	87.2 ± 13.9	0.84 (0.24, 1.43)*	0.9	86.6 ± 14.4	87.8 ± 14.1	1.06 (0.55, 1.56)*	1.4	0.56	0.54
Android fat mass (kg)	N/A	2.26 ± 1.12	-	-	N/A	2.26 ± 1.14	-	-	-	0.80
Total body fat mass (kg)	N/A	24.1 ± 9.9	-	-	N/A	24.2 ± 10.0	-	-	-	0.32
Inflammatory biomarkers										
hs-CRP[7] (mg/L)	2.89 ± 2.29	2.55 ± 1.96	-0.34 (-0.68, 0.00)*	-11.7	3.08 ± 3.07	2.85 ± 2.14	-0.23 (-0.74, 0.29)	-7.3	0.04	0.06
IL-6[7] (pg/mL)	1.65 ± 1.10	1.36 ± 0.73	-0.29 (-0.43, -0.15)*	-17.6	1.73 ± 1.08	1.39 ± 0.68	-0.34 (-0.50, -0.19)*	-19.9	0.77	0.70
Adiponectin[7] (μg/mL)	8.98 ± 4.71	8.97 ± 4.36	0.00 (-0.38, 0.37)	0.0	8.91 ± 4.61	8.81 ± 4.23	-0.09 (-0.41, 0.22)	-1.0	0.37	0.49

[1] Values are means ± SD or mean Δ post- *vs.* pre-diet (95% CI) with the corresponding % change. *hs*-CRP, high-sensitivity C-reactive protein; N/A, unavailable data. [2] * = Significant within-diet effects ($P \leq 0.05$), as determined by the least-square means statistic in MIXED models performed on delta scores representing post- *vs.* pre-diet variations. [3] *P* values for between-diet effects, as determined by MIXED models performed on delta scores representing post- *vs.* pre-diet variations. Adjustment for potential covariates (sex, centre, and/or pre-diet values of the selected parameter) was considered only when they were found to be significant at $P < 0.05$ in the models. [4] *P* values for between-diet effects, as determined by MIXED models performed on post-diet values (end-point to end-point). Adjustment for potential covariates (sex, centre, and/or pre-diet values of the selected parameter) was considered only when they were found to be significant at $P < 0.05$ in the models. [5] Analysis of post-diet values was adjusted for pre-diet values of BMI. [6] $n = 111$ post-diet in both dietary interventions as data were missing for one participant in each diet. Analysis of delta scores was adjusted for centre only. Analysis of post-diet values was further adjusted for pre-diet values of waist circumference. [7] Analyses of delta scores were performed on post- *vs.* pre-diet variations of log-transformed data since post- *vs.* pre-diet variations of crude data were not normally distributed. Analyses of post-diet values were performed on log-transformed data. All analyses were adjusted for pre-diet values of the selected inflammatory biomarker. Analyses of delta scores and post-diet values of *hs*-CRP were further adjusted for study centre.

diets were not correlated with differences in *hs*-CRP, IL-6 or adiponectin ($r = 0.04$, $P = 0.67$; $r = 0.03$, $P = 0.73$; $r = -0.004$, $P = 0.97$, respectively). Variations in BMI within the DAIRY or CONTROL diet were also not significantly correlated with variations in *hs*-CRP, IL-6 or adiponectin ($-0.09 < r < 0.18$). The CONTROL and DAIRY diets similarly increased waist circumference compared with pre-diet values (0.9% and 1.4%, respectively, within-diet $P \leq 0.01$ for both, between-diet $P = 0.56$ for the comparison of delta scores). Post-diet waist circumference, abdominal fat mass and total body fat mass did not differ between the CONTROL and DAIRY diets ($P \geq 0.32$).

The two measures of *hs*-CRP post-diet were strongly correlated after both the CONTROL and DAIRY diets ($r = 0.89$ and 0.88, respectively, both $P < 0.0001$) (**Supplemental Figure 7.1)**. The change *vs.* baseline in *hs*-CRP differed between DAIRY and CONTROL ($P = 0.04$, **Table 7.4**). Specifically, *hs*-CRP concentrations were reduced after CONTROL *vs.* diet-specific baseline values (−11.7%, within-diet $P = 0.05$, **Table 7.4**), while they were unchanged after DAIRY (−7.3%, $P = 0.47$). Both the CONTROL and DAIRY diets reduced IL-6 concentrations compared with diet-specific baseline values (−17.6 and −19.9%, respectively, within-diet $P < 0.0001$ for both) but these changes in IL-6 as well as post-diet IL-6 concentrations did not differ between the two diets ($P \geq 0.70$). There was no between- nor within-diet difference in adiponectin concentrations (all $P \geq 0.23$).

Expression of key inflammatory genes and transcription factors in whole blood cells in response to DAIRY *vs.* CONTROL was examined in a random subsample of 53 subjects having participated in both centres. Baseline characteristics of these 53 subjects did not

differ from characteristics of the whole sample of 112 participants ($P > 0.25$, not shown). Between-diet differences in inflammatory biomarkers in this subgroup of subjects also reflected differences seen in the whole study sample (not shown). Post-diet expression levels of *IL18*, *IL1B*, *NFKB1*, *PPARA*, *SREBF2*, *TNF* and *TRAF3* did not differ between CONTROL and DAIRY (all $P \geq 0.12$) (**Supplemental Table 7.4**). However, there was an effect of the diet sequence on the relative expression level of *IL1B* ($P = 0.02$ for the interaction; not shown): it was increased after DAIRY *vs.* CONTROL in participants who consumed the CONTROL diet first ($P = 0.02$, not shown), while it remained unchanged in participants who consumed the DAIRY diet first ($P = 0.30$, not shown).

7.5 DISCUSSION

The present randomized crossover study assessed the impact of dairy consumption as part of a prudent diet on inflammation using *hs*-CRP as a primary outcome and other recognized inflammatory biomarkers as secondary outcomes. We also explored the impact of dairy consumption on the expression levels of genes acting as transcription factors and involved in the inflammatory response. Compared with diet-specific baseline values, short-term consumption of approximately 3 servings/day of commercially available dairy products had no impact on *hs*-CRP or adiponectin concentrations, but reduced IL-6 concentrations. Consumption of a control dairy-free diet reduced *hs*-CRP and IL-6 concentrations compared with diet-specific baseline values, but had no impact on adiponectin concentrations. As a result, the reduction in *hs*-CRP after the CONTROL diet was significantly greater than the small non-significant variation seen after the DAIRY diet, while variations in IL-6 and adiponectin concentrations were comparable

between the two diets. These observations are unlikely to have been confounded by changes in obesity indices, although the absence of pre-diet data for abdominal fat mass and total body fat in the present study limits our analysis and interpretation of potential variation in body fat distribution during either diet.

Results from available studies that have documented the impact of dairy consumption on inflammatory biomarkers have been inconsistent. In a recent systematic review of the literature (20), we have highlighted that only one of eight clinical studies so far was *a priori* designed for that purpose. This one study reported favorable effects of adequate dairy consumption over 12 week (> 3.5 daily servings *vs.* < 0.5 daily servings) on oxidative and inflammatory stress in individuals with metabolic syndrome (37). The biomarkers of inflammation that were favourably modified after dairy consumption in that study were *hs*-CRP, IL-6, TNF-α, monocyte chemoattractant protein 1 (MCP-1) and adiponectin. In the seven other randomized trials discussed in the systematic review, change in the inflammatory profile was assessed as a secondary or unclearly defined outcome (35, 38-42). Three of these seven studies reported inflammation-lowering effects of dairy consumption (35, 39). In the two studies reported by Zemel *et al.* in (39), concomitant reductions in adiposity indices such as total body fat and trunk fat after consumption of dairy products are likely to have confounded their impact on the inflammatory profile (43, 44). On the other hand, Zemel *et al.* (35) also documented the short-term effects of a dairy-rich diet compared with soy products on inflammatory stress in overweight and obese subjects (n = 20) in the absence of adiposity changes. The dairy-based diet had significant anti-inflammatory effects as evidenced by reductions in *hs*-CRP, IL-6, TNF-α and MCP-1 concentrations as well as an increase in adiponectin

concentrations. These effects were evident by day 7 of the dairy-based diet and magnified at the end of the 28-day treatment period. The study length and crossover design were similar to features of the present study. However, the dairy-rich diet in Zemel *et al.* (35) incorporated smoothies formulated with non-fat dry milk, which does not reflect consumption of commercially available dairy products as per our own study. The extent to which differences in dairy product formulation between the two studies may have influenced the bioavailability of nutrients in dairy and, consequently, their impact on inflammation is unknown.

The purported benefit of dairy consumption on inflammation has not been observed consistently (38, 40-42). Van Meijl and Mensink (42, 45) reported no effect of an 8-week low-fat dairy diet *vs.* a control diet on CRP and IL-6 among 40 overweight or obese subjects. This study shares several features with our study, including the crossover design, the use of CHO-rich control products (fruit juice and biscuits) and the relatively short-term duration of the diets. The lack of effect of dairy consumption on *hs*-CRP is also consistent with our findings.

Exploratory analyses in a subsample of study participants showed that consumption of 3 servings of dairy products per d had no impact on expression levels of key inflammatory genes and transcription factors in whole blood cells compared with a dairy-free diet. Consistent with our results, in a randomized parallel controlled feeding study, Van Loan *et al.* (46) showed no change in relative transcript abundances of inflammatory genes such as TNF-α, IL-6, MCP-1 (also known as CCL2), and IL-1β in subcutaneous adipose tissue of overweight and obese adults consuming energy-restricted diets combined with

either adequate dairy intake (3-4 servings/day) or low dairy intake (≤ 1 serving/day). They also showed no between-diet difference in circulating concentrations of inflammatory mediators including pro-inflammatory cytokines, *hs*-CRP and adiponectin. To the best of our knowledge, this is the only other report having assessed inflammatory gene expression levels following dairy consumption.

Limitations and strengths of this intervention trial need to be pointed out. First, in contrast with some of the previous studies, which matched macronutrient proportions between experimental diets (35, 37, 39, 41), the present study had by design inevitable but important differences in macronutrient proportions between the CONTROL and DAIRY diets. However, analyses of self-reported nutritional intakes showed that differences in global dietary intakes between the CONTROL and DAIRY diets were almost entirely attributable to foods provided during each phase of the intervention rather than to differences in participants' background diet between each phase (**Supplemental Table 7.3**). The higher consumption of "fruits" in DAIRY *vs.* CONTROL represented the major difference in participants' background diet, but it was mostly explained by a slightly higher intake of fruit juice while on DAIRY (not shown). The lack of diet-specific baseline data on food intake limits analysis and interpretation of dietary change when participants were on the CONTROL and DAIRY diets. This is also the case for gene expression levels, which were not measured at baseline of each dietary phase. It is stressed that the CONTROL diet had a beneficial impact on pro-inflammatory markers compared with diet-specific baseline values. A possible explanation for this effect is that control foods may have provided bioactive ingredients such as vitamins and phytochemical compounds, which have been suggested to exert potential anti-

inflammatory effects in some (47-49), but not all studies (50-52). However, we argue that the use of commercially-available control products was appropriate and justified, each representing a plausible alternative to dairy products when transposed into a non-experimental, real-life setting. Both diets diet were consumed over a 4-week period. This relatively short time frame was sufficient to observe significant reductions in IL-6 concentrations from baseline values. It is also stressed that two randomized controlled trials previously reported significant changes in inflammatory biomarkers even after shorter periods, i.e. after only one week of intervention with dairy products (35, 37). However, whether longer-term dairy consumption triggers a different anti-inflammatory response remains to be addressed. Key strengths of the present study include the randomized crossover and multicentre design, the large number of subjects enrolled, the fact that participants were specifically selected to have subclinical inflammation, as well as the high compliance of subjects to the experimental procedures. The fact that inflammatory biomarkers concentrations were measured twice at the end of each diet to minimize intra-individual variations is also a significant strength. This is the first diet study, to the best of our knowledge, having used such an approach. The free-living nature of the protocol and the use of commercially available products make it relevant to everyday clinical practice.

In conclusion, we showed that short-term dairy consumption in the context of a prudent diet has no significant impact on *hs*-CRP or adiponectin concentrations, but reduces circulating levels of the pro-inflammatory cytokine IL-6 from baseline values. Our study was not able to relate any of these changes to variations in expression levels of key inflammatory genes in whole blood cells compared to a dairy-free diet in healthy men

and women with low-grade systemic inflammation. Short-term dairy consumption was also associated with a slight increase in BMI, but this did not correlate with variations in the inflammatory profile. Combined with data from previous work, these results confirm that consumption of a combination of low- and high-fat dairy products as part of a healthy diet has no adverse effects on inflammation (20). Whether dairy consumption exerts anti-inflammatory effects is currently not fully substantiated by existing studies on this topic and requires further demonstration through well-designed and adequately powered studies of perhaps longer duration as well.

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Conflict of interest statement is as follows: MCV has received funding in the past from Danone Inc. PJ has received funding from the Dairy Farmers of Canada and Danone Inc. PC and BL have received funding in the past from the Dairy Farmers of Canada, Dairy Australia and Agriculture and Agri-Food Canada. BL also has received funding from Danone Institute and he is the Chair of the Expert Scientific Advisory Committee (ESAC) that reviews funding applications as part of the peer-reviewed research funding program of Dairy Farmers of Canada. MEL, AC, MMA, and MCL have no conflicts of interest.

The authors' responsibilities were as follows: BL, PC and PJ designed the study. PC was responsible for the screening and medical supervision of the study participants. MCV contributed her expertise for the gene expression studies. MCL and MMA coordinated the study with the collaboration of AC. MEL and AC performed statistical analyses, interpreted the data, and wrote the manuscript. BL had primary responsibility for final content. All authors critically reviewed the manuscript and approved its final version.

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7.8 ONLINE SUPPORTING MATERIAL

Supplemental Table 7.1 GenBank and Life Technologies numbers of the analyzed genes[1]

	# GenBank[2]	# Life Technologies[3]
Target genes		
CCL2	NM_002982	Hs00234140_m1
IL18	NM_001562	Hs01038788_m1
IL6	NM_00600	Hs00985639_m1
IL1B	NM_000576	Hs01555410_m1
NFKB1	NM_003998	Hs00765730_m1
NPR3	-	Hs01099013_m1
PPARA	NM_005036	Hs00947536_m1
SREBF2	NM_004599	Hs01081784_m1
TNF	NM_000594	Hs01113624_g1
TRAF3	NM_145725	Hs00936781_m1
Control genes		
G6PD	-	Hs00166169_m1
GAPDH	NM_002046	Hs00266705_g1

[1] *G6PD*, glucose-6-phosphate dehydrogenase; *NPR3*, natriuretic peptide receptor C. [2] National Center for Biotechnology Information (NCBI) GenBank (http://www.ncbi.nlm.nih.gov/genbank/). [3] Life Technologies Genome Database (http://www.lifetechnologies.com/order/genome-database/)

Supplemental Table 7.2 Energy and nutrient intakes prior to (USUAL) as well as during the CONTROL and DAIRY dietary interventions in men and women with low grade inflammation[1]

Nutrient	USUAL	CONTROL	DAIRY	*P*[2]
Energy (kJ)	8056 (3840)	7321 (3506)[a]	7856 (3048)[b]	0.0002
Alcohol (%)	1.0 (2.3)	0.8 (1.8)	0.7 (1.7)[a]	<0.0001
Lipids (%)	33.9 (6.3)	33.0 (7.7)	30.3 (6.1)[a]	0.0004
SFA	11.0 (3.1)	8.7 (2.4)[a]	10.7 (2.3)[b]	<0.0001
MUFA	13.1 (3.3)	14.1 (3.4)[a]	11.8 (3.0)[a,b]	<0.0001
PUFA	6.2 (2.3)	7.2 (1.7)[a]	5.2 (1.8)[a,b]	<0.0001
TFA[3]	1.3 (0.5)	1.1 (0.6)	1.1 (0.5)[a]	0.008
Dietary cholesterol (mg)	242 (128)	174 (124)[a]	209 (130)[a,b]	<0.0001
Protein (%)	17.5 (4.3)	14.0 (3.5)[a]	18.5 (3.1)[a,b]	<0.0001
Carbohydrate (%)	49.8 (7.7)	55.1 (7.9)[a]	52.3 (8.4)[a,b]	<0.0001
Fibre (g)	22.0 (12.2)	22.6 (14.5)	21.0 (13.8)[a,b]	0.02
Sodium (mg)	2665 (1273)	2239 (973)[a]	2357 (1189)[a]	<0.0001
Calcium (mg)	1009 (769)	437 (249)[a]	1411 (398)[a,b]	<0.0001
Vitamin D (μg)	7.7 (6.0)	3.7 (4.7)[a]	8.3 (5.5)[a,b]	<0.0001

[1] Values are medians (IQR). n = 108 for the USUAL intakes (i.e. measured at screening) as dietary data for 4 participants were excluded because of non-plausible energy intakes (< 2092 or > 14 644 kJ [< 500 or > 3500 kcal] in women and < 3347 or > 17 573 kJ [< 800 or > 4200 kcal] in men); n =106 in the CONTROL diet as dietary data from the FFQ were missing for 3 participants and 3 participants were excluded because of non-plausible energy intakes; n = 104 in the DAIRY diet as dietary data were missing for 6 participants and 2 participants were excluded because of non-plausible energy intakes. [2] P values for differences between measurements, as determined by the Friedman test. Pairwise comparisons were performed using rank transformation of the data followed by the general linear model procedure and the Tukey adjustment for multiple comparisons. [a] = significantly different from USUAL ($P < 0.05$); [b] = significantly different from CONTROL ($P < 0.05$). [3] TFA, *trans* fatty acids.

Supplemental Table 7.3 Nutritional intakes during the CONTROL and DAIRY dietary interventions in men and women with low grade inflammation after exclusion of foods provided to participants during each phase[1]

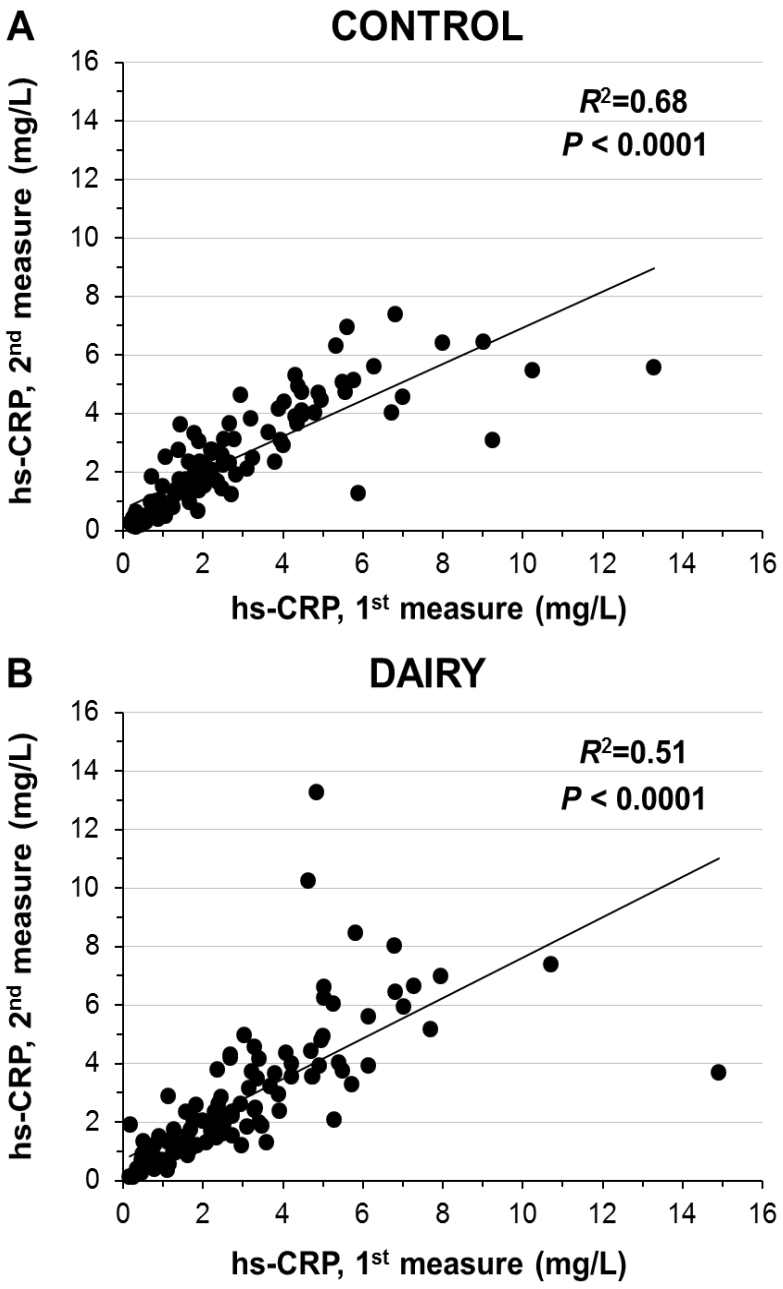
	CONTROL	DAIRY	*P*[2]
Food groups	*servings/day*		
Dairy products	0.0 (0.3)	0.1 (0.2)	0.72
Fruits and vegetables	4.8 (4.2)	5.5 (5.1)	0.0003
Fruits	1.6 (2.2)	2.4 (3.0)	0.0002
Vegetables	2.7 (2.8)	2.7 (3.0)	0.10
Grain products	3.7 (2.8)	4.1 (2.5)	0.28
Meat and alternatives	2.0 (1.6)	1.9 (1.8)	0.69
Animal protein	1.5 (1.2)	1.5 (1.2)	0.17
Vegetable protein	0.3 (0.6)	0.4 (0.6)	0.11
Nutrients			
Energy (kJ)	5237 (3117)	5903 (2974)	0.009
Alcohol (%)	0.9 (2.4)	0.9 (2.4)	0.17
Lipids (%)	32.8 (8.3)	30.8 (8.1)	0.48
SFA	8.0 (2.6)	8.1 (2.5)	0.36
MUFA	13.9 (5.2)	12.8 (4.2)	0.15
PUFA	6.9 (2.3)	6.7 (2.3)	0.54
TFA[3]	1.2 (0.7)	1.2 (0.6)	0.76
Dietary cholesterol (mg)	164 (126)	161 (120)	0.26
Protein (%)	16.2 (4.1)	15.4 (3.6)	0.01
Carbohydrate (%)	51.3 (10.3)	53.9 (10.9)	0.03
Fibre (g)	18.4 (13.4)	20.4 (13.6)	0.06
Sodium (mg)	1652 (914)	1845 (1187)	0.051
Calcium (mg)	343 (208)	373 (236)	0.09
Vitamin D (μg)	3.7 (4.7)	2.8 (4.3)	0.26

[1] Values are medians (IQR). Data represent nutritional intakes without the contribution of fruit juice, vegetable juice, nuts and cookies during the CONTROL diet and without the contribution of milk, yogurt and cheese during the DAIRY diet. *n* =106 in the CONTROL diet as dietary data from the FFQ were missing for 3 participants and 3 participants were excluded because of non-plausible energy intakes (< 2092 or > 14 644 kJ [< 500 or > 3500 kcal] in women and < 3347 or > 17 573 kJ [< 800 or > 4200 kcal] in men); *n* = 104 in the DAIRY diet as dietary data were missing for 6 participants and 2 participants were excluded because of non-plausible energy intakes. [2] *P* values obtained by the Wilcoxon matched-pairs signed-rank test. [3] TFA, *trans* fatty acids.

Supplemental Table 7.4 Mean fold change in expression levels of key inflammatory genes and transcription factors after the DAIRY compared with the CONTROL diet in a random subset of 53 men and women with low grade inflammation[1]

Genes	Fold change (DAIRY *vs.* CONTROL)[2]	*P*[3]
IL18	1.09	0.20
IL1B	1.04	0.34
NFKB1	1.05	0.12
PPARA	1.04	0.46
SREBF2	1.05	0.36
TNF	1.00	0.98
TRAF3	1.05	0.32

[1] *G6PD*, glucose-6-phosphate dehydrogenase; *IL18*, interleukin 18; *IL1B*, interleukin 1-beta; *NFKB1*, nuclear factor kappa-B subunit 1; *PPARA*, peroxisome proliferator-activated receptor alpha; *SREBF2*, sterol regulatory element binding transcription factor 2; *TNF*, tumor necrosis factor; *TRAF3*, TNF receptor-associated factor 3. [2] Values are fold change in gene expression calculated using the $2^{-\Delta\Delta Ct}$ method, i.e. 2^(mean ΔCt from the DAIRY diet – mean ΔCt from the CONTROL diet). ΔCt were calculated as Ct values of a selected target gene – Ct values of the internal control gene *G6PD*. Ct values used in the formula consisted of the mean of the triplicates' individual Ct values, unless the SD of a triplicate was > 0.5, in which case the outlier value was excluded from the calculation of the mean. [3] *P* values for between-diet effects, as determined by MIXED models performed on post-diet gene expression levels normalized to the expression of the reference gene *G6PD*. Adjustment for potential covariates including sex, age, and study centre did not change the results.



Supplemental Figure 7.1 Correlation analysis between the two post-diet measures of high-sensitivity C-reactive protein (*hs*-CRP) in the CONTROL (A) and DAIRY (B) diets

CHAPTER VIII

GENERAL DISCUSSION

8.1 SUMMARY AND IMPLICATIONS

The observations of studies encompassed in this thesis have important implications for human health and management of cardiovascular disease (CVD) in relation to the recommended level of dairy consumption. Diet is a well-defined modifier of disease risk. Given that milk and dairy products are staple components of most Western diets, and are recommended for consumption by food guides, it is important to understand whether these foods increase or decrease the risk of chronic disease, including their effects on surrogate endpoint measures. Circulating cholesterol concentrations are an established risk factor for CVD and a major target of public health nutrition policies (1, 2). A 10% reduction in serum total cholesterol (TC) concentrations has been associated with 20% to 50% lower risk of coronary heart disease (CHD), and a 25-30% reduction in CHD-related mortality over long terms (3). Similarly, a 10% reduction in low-density lipoprotein cholesterol (LDL-C) concentrations has been associated with 20% reduction in cardiovascular events (3). As part of an overall healthy diet, Canada's Food Guide recommends 2-3 servings/day intake of dairy (4). Although not without exception, dairy intake in epidemiological studies appears to provide some reduction in risk of CVD incidence. Similarly, findings from some but not all dietary interventions underscore beneficial impacts of individual dairy product intake on risk factors for CVD, such as circulating cholesterol and inflammatory biomarker concentrations. While the evidence is still controversial and needs further understanding, the implications for dairy consumers

are potentially very substantial. Data from the present research demonstrate a range of changes in serum cholesterol and cholesterol metabolism responses that are largely modulated by an inter-individual variability following the intake of a dairy diet.

A wealth of knowledge exists on dietary fatty acids (FAs) in relation to cholesterol concentrations and CVD risk. Dairy products contain over 400 different types of FAs (5), some of which are known to associate with favourable and others with unfavorable effects (6, 7). Given the gap of knowledge in research examining the degree to which dairy fat intake is reflected in the circulating FAs, potentially affecting cholesterol concentrations, results in Chapter IV of this thesis have important implications. There, in agreement with data from observational studies (8-15), intake of the dairy diet modifies FA profile in a manner that enables the detection of two established biomarkers of milk fat intake, C15:0 and C17:0, by ~18% and ~8%, respectively. These FAs have been previously associated with positive (12, 15, 16) and, in one case, negative (17) cardiovascular impacts.

The present research enhances our understanding of dietary and genetic factors that regulate cholesterol metabolism. Specifically, in accordance with some (18-20) but not all (21, 22) previous studies, the recommended intake of dairy versus control products are herein shown to influence serum TC and LDL-C concentrations in a likely non-clinically significant range of +0.08 to +0.10 mmol/L. More importantly, the magnitude of these responses appears to associate with genetic variations within key genes along the cholesterol metabolic pathways, including *ABCG5*, *CYP7A1*, and *DHCR7* (Chapter V). Data from Chapter VI further demonstrate that the dairy intake in the present research is

associated with changes on the cholesterol absorption and biosynthesis levels, as measured in plasma using stable isotope techniques. Again, this association appears to have a genetic basis in the presence of common polymorphisms within the *CYP7A1* and *DHCR7* genes. These observations of a health-modulating genetic heterogeneity are important in establishing population-wide recommendations for dairy products over the long run in that they could help identifying individuals who could benefit the most from consuming dairy diets. This supports the promising concept of personalized nutrition and health assessment.

In addition, CVD is a complex condition that involves several physiologic systems, therefore, targeting multiple rather than individual risk factors of the disease is likely to result in better clinical outcomes. Systemic inflammation characterized by elevated circulating concentrations of inflammatory biomarkers, such as C-reactive protein (CRP) (>3.0 mg/L), is an emerging risk factor for CVD (23). Although analysis of within-diet changes (post- *vs.* pre-diet values) failed to reveal a reduction in CRP levels following the intake of dairy products in the present work (−7.3%, $P = 0.47$), dairy may still be targeting inflammation through a reduction in interleukin (IL)-6 concentrations (−19.9% post- *vs.* pre-diet, $P < 0.0001$), an independent risk factor for CVD (24, 25). In any case, the totality of observations herein suggests that a short-term daily consumption of a combination of low-fat and regular dairy products, as part of a healthy diet, has no adverse effects on inflammation in apparently healthy individuals.

8.2 LIMITATIONS AND FUTURE DIRECTIONS

Some limitations are acknowledged to exist in this research, which could have led to type I (false positive) or type II (false negative) errors. Firstly, although the multicentre free-living design with the use of commonly-consumed dairy products was chosen to best reflect a real life scenario, this design is not the 'gold standard' for evaluating health outcomes in dietary interventions (26). Fully-controlled, randomized, crossover designs where all meals are provided to participants and study treatments consumed under close supervision offer better insights into the potential impact of dairy intake, leading to more accurate conclusions in future studies. Interrelated with this point, the dairy and control products of the present research were by nature completely different in macro- and micronutrient proportions, limiting the ability to identify the precise dietary factors of effect and limiting the generalizability of findings. For instance, when dairy is replaced by food products other than those constituting the control of the present work, what effects ensue? Also, analyzing FA profile of the study products would have been helpful in establishing more accurate associations between dietary intakes and plasma FA concentrations.

Secondly, the circulating lipid concentrations were not assessed at baseline in every study individual and data presented for the endpoint measures were based on a single blood sampling. Given that lipid profiles naturally experience day-to-day fluctuations, a better estimation of effect would have been achieved through repeated sampling and calculations of means. This is also true for the inflammatory gene expressions, which were not measured at baseline of each dietary phase.

Thirdly, out of 124 individuals who completed the study, n = 101 were genotyped for associations with serum cholesterol concentration and metabolic responses. Although this population size is larger than most similar dietary interventions and detected, or in case of *CYP7A1* actually replicated previous findings of, some gene-diet interactions, it is still considered small when compared to genome-wide association studies (GWASs). In this context, unevenly distributed frequencies of genetic variants within the single nucleotide polymorphisms (SNPs) under study were often observed. Therefore, the findings of novel associations in this research would need replication in full-feeding clinical trials of larger sizes. Future studies should also aim for examining associations of dairy diets with SNPs across genes in regulatory pathways that control other biomarkers of cardiovascular health, such as FA and inflammation homeostasis.

Fourthly, considering that we took a candidate gene approach, only a limited number of systematically-chosen SNPs within certain genes were studied in the present research. Of note, however, failure to detect an association with these SNPs does not exclude the possibility that other SNPs in the candidate genes are related to cholesterol metabolism following the dairy intake. This example of a potential type II error can be corrected through the use of next-generation DNA sequencing (27) in future studies where the entire genes of interest could be examined thoroughly (28).

Considering these limitations, appropriately-designed future dietary interventions looking into the genetic heterogeneity in biomarker responsiveness to dairy diets ought to recruit for carriers of genotypes that have already been associated with clear phenotypic responses to dairy (i.e. hyper- *vs.* hypo-responders). Although not without possible type I

errors, the use of next-generation DNA (or whole genome) sequencing is another fruitful area of research where, unlike the candidate SNP approach, it would eliminate bias in the selection of genes and SNPs of interest.

8.3 FINAL CONCLUSIONS

Growing prevalence of CVD, a globally leading cause of morbidity and mortality in the past few decades, has been accompanied by escalating costs related to healthcare and society's loss of productivity, putting this diet-related disorder among the world's top public health policy priorities (29). Deregulated cholesterol metabolism, including hypercholesterolemia, is a well-defined biomarker of CVD. Management of circulating cholesterol concentrations through lifestyle modifications, such as adherence to healthy dietary habits and physical activity, and avoidance of excessive alcohol consumption and smoking, is a foremost strategy to target lower CVD risk (1, 2). Dietary habits consistent with guidelines for healthy eating have the potential to produce substantial health and economic benefits (30). Such guidelines commonly center around consumption of plant-based foods, including fruits and vegetables, whole grains, and legumes, together with low-fat dairy, lean protein meat including seafood, and "heart-healthy" dietary oils to improve population health and wellness (31). Similar to most industrialized countries, Canada's Food Guide recommends a dairy consumption in the level of 2-3 servings/day range for adults, as part of an overall healthy lifestyle, for optimal nutritional and health status (4). The work presented in this thesis provides new evidence in regards to impacts of the recommended intake of commonly-consumed dairy products in Canada on traditional and emerging biomarkers of cardiovascular health.

Through a selection of well-established techniques tackling several mechanistic levels of action, the totality of studies in this thesis for the first time reveal an important contribution of the genomic architecture in the responsiveness of established biomarkers of health to dairy-based diets. Specifically, the present research suggests that common genetic variants along known candidate cholesterol metabolic pathways, including those related to bile acid flux, are involved in determining responses of serum cholesterol, as well as cholesterol absorption and biosynthesis rates, in healthy adults, to the recommended intake of dairy products in Canada. The observations could explain at least part of the inconsistency of evidence in the literature regarding milk and dairy consumption in relation to cholesterol concentrations and cardiovascular health. Given the increased promotion and popularity of a variety of dairy products in the marketplace, and given the advantage of evaluating impacts of dietary patterns versus single nutrients on complex disorders, the current thesis findings represent an important step towards the promising era of personalized nutrition and health assessment. In the long run, the use of genetic markers for disease risk prediction in responders and non-responders to dietary intakes, such as dairy, is expected to contribute to better understanding, and management, of the social and economic burdens of diet-related epidemics in Canada and around the world.

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APPENDICES

APPENDIX I

ETHICS APPROVAL FOR STUDIES CORRESPONDING TO CHAPTERS IV, V, VI, VII

UNIVERSITY OF MANITOBA

BANNATYNE CAMPUS
Research Ethics Boards

P126-770 Bannatyne Avenue
Winnipeg, Manitoba
Canada R3E 0W3
Tel: (204) 789-3255
Fax: (204) 789-3414

APPROVAL FORM

Principal Investigator: Dr. P. Jones
Sponsor: Dairy Farmers of Canada

Ethics Reference Number: B2010:162
Date of REB Meeting: November 22, 2010
Date of Approval: January 21, 2011
Date of Expiry: November 22, 2011

Protocol Title: Impact of Dairy Consumption on Inflammation: A Clinical Study

The following is/are approved for use:

- **Proposal, Version dated 05/11/2010 with amendments outlined in letter dated January 10, 2011**
- **Research Subject Information and Consent Form, Version 2 dated 10/01/2011**
- **Study Instruments submitted November 5, 2010:**
  - **Diet records**
  - **3-D Physical Activity Diary**
  - **24-H Food Recall**
  - **Study Measures**
  - **Blood Sample: Checklist**
  - **Eligibility Chart Assessment form**
  - **General Information Assessment form**
  - **Medical Lifestyle Questionnaire (screening)**
  - **Medical Lifestyle Questionnaire (Weeks 0/4/8/12)**
  - **Medical Lifestyle Questionnaire (Weeks 2/10)**
  - **Medical Lifestyle Questionnaire (Weeks 3/11)**
  - **Questionnaire on Side Effects**
  - **Termination Assessment form**
- **Poster submitted November 5, 2010**

The above was approved by Dr. Nicholas Anthonisen, Chair, Biomedical Research Board, Bannatyne Campus, University of Manitoba on behalf of the committee per your letter dated January 10, 2011. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations of Canada.*

This approval is valid for one year from the date of the meeting at which it was reviewed. A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval should be sought from the relevant institution, if required.

Sincerely yours,

[redacted]

Nicholas Anthonisen, MD, Ph.D
Chair,
Biomedical Research Ethics Board
Bannatyne Campus

Please quote the above Ethics Reference Number on all correspondence.
Inquiries should be directed to the REB Secretary **Telephone**: (204) 789-3255/ **Fax:** (204) 789-3414

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APPROVAL FORM

Principal Investigator: Dr. P. Jones
Sponsor: Diary Farmers of Canada

Ethics Reference Number: B2010:162
Date of Approval: March 1, 2011

Protocol Title: Impact of Dairy Consumption on Inflammation: A Clinical Study

The following is/are approved for use:

- **Protocol, Version dated February 10, 2011**
- **Research Subject Information and Consent Form, Version 3 dated 09/02/2011**

The above was approved by Dr. Nicholas Anthonisen, Chair, Biomedical Research Board, Bannatyne Campus, University of Manitoba on behalf of the committee as per your submission dated February 10, 2011. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations of Canada.*

A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval should be sought from the relevant institution, if required.

Sincerely yours,

[redacted]

Nicholas Anthonisen, MD, Ph.D
Chair,
Biomedical Research Ethics Board
Bannatyne Campus

Please quote the above Ethics Reference Number on all correspondence.
Inquiries should be directed to the REB Secretary **Telephone**: (204) 789-3255/ **Fax:** (204) 789-3414

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APPROVAL FORM

Principal Investigator: Dr. P. Jones
Sponsor: Dairy Farmers of Canada

Ethics Reference Number: B2010:162
Date of Approval: July 19, 2011

Protocol Title: Impact of Dairy Consumption on Inflammation: A Clinical Study

The following is/are approved for use:

- **Protocol, Version 3 dated July 06, 2011**
- **Research Subject Information and Consent Form, Version 4 dated 06/07/2011**

The above was approved by Dr. Lindsay Nicolle, Chair, Biomedical Research Board, Bannatyne Campus, University of Manitoba on behalf of the committee as per your submission dated July 8, 2011. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations of Canada.*

A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval should be sought from the relevant institution, if required.

Sincerely yours,

[redacted]

Lindsay Nicolle, MD, FRCPC
Chair,
Biomedical Research Ethics Board
Bannatyne Campus

Please quote the above Ethics Reference Number on all correspondence.
Inquiries should be directed to the REB Secretary **Telephone**: (204) 789-3255/ **Fax:** (204) 789-3414

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APPROVAL FORM

Principal Investigator: Dr. P. Jones
Sponsor: Dairy Farmers of Canada

Ethics Reference Number: B2010:162
Date of Approval: June 12, 2012
Date of Expiry: November 22, 2012

Protocol Title: Impact of Dairy Consumption on Inflammation: A Clinical Study

The following is/are approved for use:

- **Annual Approval**
- **Research Subject Information and Consent Form, Version 4 dated 06/07/2011**
- **Research Subject Information and Consent Form – Additional Consent Form for Genetic Analysis, Version 1 dated 08/05/2012**

The above was approved by Dr. Lindsay Nicolle, Chair, Biomedical Research Board, Bannatyne Campus, and University of Manitoba on behalf of the committee per your submission dated June 5, 2012. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations of Canada.*

This approval is valid until the expiry date only. A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval should be sought from the relevant institution, if required.

Sincerely yours,

Lindsay Nicolle, MD, FRCPC
Chair,
Biomedical Research Ethics Board
Bannatyne Campus

Please quote the above Ethics Reference Number on all correspondence.
Inquiries should be directed to the REB Secretary **Telephone**: (204) 789-3255/ **Fax:** (204) 789-3414

www.umanitoba.ca/medicine/ethics

APPENDIX II

FORMS CORRESPONDING TO STUDIES DESCRIBED IN CHAPTERS IV, V, VI, AND VII

Study Advertisements – Poster

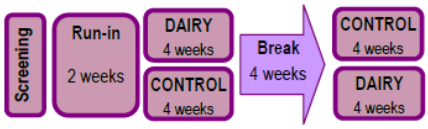
General Information Sheet

Unofficial General Information Sheet

The Impact of Dairy Intake on Health : A Nutrition Study

Study objective To assess the impact of dairy consumption on multiple risk factors for disease; such as high blood cholesterol levels and inflammatory markers. The intake of dairy, in a healthy diet, may favourably affect a number of common health indicators.

Study design This study is open for generally healthy men and women, age 18-70, who do not take lipid, blood pressure, or inflammatory medications.



The study will consist of 2 dietary phases, DAIRY and CONTROL (28 days each), separated by a one-month break, during which time you will return to your normal eating habits. Throughout each phase, you will visit the RCFFN once every week to undergo some health measures and/or obtain dairy (reduced-fat milk, low-fat yogurt, regular cheddar cheese) or control (juice, cookies, cashews) products. You will be asked to incorporate 3 servings of these foods in your diet, and will receive dietary guidance for the study period. In screening for this study, you will have 2 "fasting" blood tests and a brief physical exam to confirm your health status and eligibility.

Study measurements At the beginning and end of each dietary phase, a fasting (nothing to eat or drink for 12 hours) blood sample will be collected between 6:30 and 9:00 am. This is to measure your cholesterol levels, and other key nutrition/health values. The total amount of blood drawn each time is approximately 2 tablespoons.

A special, optional, test will take place during days 24-27; and you may participate in it. On day 24, you may be asked to consume a small quantity of "labelled" cholesterol mixed in a teaspoon of margarine on half an English muffin. This labelled cholesterol is nearly identical to the regular cholesterol but can be distinguished and traced in blood. A fasting blood sample (~1 tablespoon) will be collected before the intake of this meal and in the following 3 mornings. On day 27, after blood draw, you will also consume 3 tablespoons of tagged water. This test does not pose any health risk, and will allow us to find out how much dietary cholesterol your body absorbs and how much cholesterol it produces. Fecal samples may be collected during this test.

Body measures (weight, height, waist circumference) and blood pressure will be recorded regularly. At the beginning and end of each dietary phase, as well, you will undergo non-invasive tests to assess the amount of fat in your body and examine the health of your blood vessels. These tests will take approximately an hour to complete. We may also invite you to measure the number of calories that your body burns before and after eating a set meal. In addition, you will be asked to fill out dietary and physical activity records, and will be financially compensated for your participation.

Thank you for taking time to read this information and considering to volunteer for our study. For more information, please call us at 298-5483.

Subject Consent Form

UNIVERSITY OF MANITOBA

196 Innovation Drive, Smartpark
University of Manitoba, Winnipeg, MB R3T 2N2
Tel: 204.298.5483 Fax: 204.474.7552

Richardson Centre for Functional Foods and Nutraceuticals

RESEARCH SUBJECT INFORMATION AND CONSENT FORM

Title of Study: **The Impact of Dairy Consumption on Inflammation: A Clinical Study**

Principal Investigator: **Peter Jones, PhD**
Richardson Centre for Functional Foods and Nutraceuticals (RCFFN)
University of Manitoba
196 Innovation Drive, Smartpark
Winnipeg, Manitoba R3T2N2
Phone: (204) 474-8883

Sponsor: **Dairy Farmers of Canada**
21 Florence Street
Ottawa, ON K2P 0W6
Phone: (613) 236-9997, fax: (613) 236-0905
Email: info.policy@dfc-plc.ca

You are being asked to participate in a research study. Please take your time to review this Information and Consent Form and discuss any questions you may have with the study staff. You may take your time to make your decision about participating in this clinical trial and you may discuss it with your regular doctor, friends and family. This consent form may contain words that you do not understand. Please ask the study doctor or study staff to explain any words or information that you do not clearly understand.

Purpose of Study

The purpose of this nutrition study is to evaluate the impact of dairy consumption on the heart health and risk factors, mainly inflammatory markers, for chronic diseases. Systemic inflammation is a key defense mechanism that is widely linked to many disorders such as diabetes, heart disease, and cancer. In this study, we primarily aim at finding out if dairy in our diet decreases the likelihood of having a higher degree of inflammation in blood. The past few years have provided evidence that the intake of some nutrients in dairy may result in favorable modifications on the inflammatory status. We, therefore, anticipate that the consumption of dairy products per se will improve the inflammatory profile in our bodies, and other health-related indicators. This study is not a dieting plan, and it does not involve restricting intake of calories for weight loss.

Study Procedures

This study is, initially, open for healthy men and women, aged 18-70. You may not be eligible if you smoke, experienced variations in body weight in the past 2 months, are anaemic, aim to lose weight, currently

Initials of Subject: _____

breastfeed, routinely take medications for high cholesterol, hypertension, or inflammation, or have allergies to dairy, egg, or cashews.

If you agree to join this Study, you will be asked to visit our Centre to give a fasting (nothing to eat or drink for 10-12 hours) blood sample of about 1 teaspoon. This is to measure inflammatory markers and lipid profile. In this visit, we will measure your height, weight, waist girth, hip girth, and blood pressure. If you meet the eligibility criteria, you will be asked to complete a 3-day food diary, a 3-day physical activity journal, and a web food frequency questionnaire before the Study begins.

Depending on your dietary habits, we may allow a 2-week "run-in" period prior to the start date to help you get familiar with the Study's dietary recommendations for healthy eating. The Study will consist of 2 phases, each for 4 weeks, during which time you will be provided with dairy or energy-equivalent products weekly. You will be asked to incorporate 3 items of these in the eating routine that we will recommend. A washout period of 4 weeks will separate phase I and II. The two dietary phases will include:

1) The *DAIRY* diet, where you will be asked to incorporate 3 servings of dairy into your everyday diet. These will be in the form of each of the followings, per day: reduced fat milk (1% fat, 375 ml), low fat yogurt (1.5% fat, 175 g), and cheese (regular cheddar, 30 g).
2) The *CONTROL* diet, where you will be asked to consume energy-equivalent products. Milk will be replaced by fruit and vegetable juice. Yogurt will be replaced by desserts (1 cookie per day). And, cheese will be replaced by cashews (20 g per day).

This study is with a "crossover" design which means that you will receive both of the 2 diets described above, *DAIRY* and *CONTROL;* however, the order will be unknown (such as *DAIRY-CONTROL* or *CONTROL-DAIRY*). In an emergency, this information will be made available.

The Study design and sequence of tests is provided below. You will be asked to visit our Centre once every week, for 10 weeks, to obtain the dairy and control products, and undergo some measures.

On **weeks 0, 3, and 4** of each phase, a fasting blood sample (~2 tablespoons) will be collected. Each blood draw will take about 5 minutes, and the total amount of blood drawn for the entire study will approximately be 12 tablespoons.

On **weeks 0 and 4**, we will measure your height, weight, waist girth, hip girth, and blood pressure.

On **week 2**, we will measure your height, weight, waist girth, hip girth, and will ask you to tell us what you ate and drank in the previous day.

At the end of each phase (**week 4**), we will ask you to fill out a 3-day food record, a 3-day physical activity journal, and to complete a web food frequency questionnaire. Also, we will measure your total body composition, including the amount of body fat, and examine the health of your blood vessels, using non-invasive tools called DXA and EndoPAT, respectively. For DXA, you will lie on your back while a scan arm passes from your head to feet. This test will take ~30 minutes to complete. The EndoPAT test will take 30-40 minutes to complete, during which time probes will be placed on your fingertips.

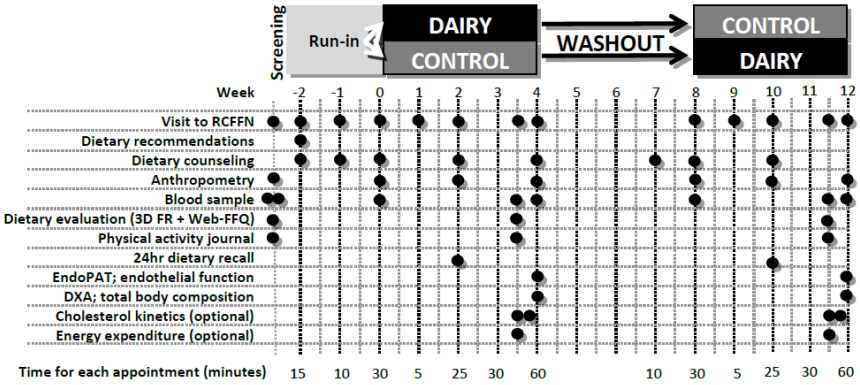
Initials of Subject: _____

Impact of Dairy Consumption on Inflammation: A Clinical Study

During **weeks 1** and **4**, we may invite you to measure the number of calories that your body burns before and after eating a set meal. This will be done by having you lie comfortably in bed while wearing a large clear "bubble" over your head for 30 minutes, right before breakfast and every 30 minutes thereafter, for a total of 5.5 hours. The bubble is ventilated, lightweight, and comfortable (you can breathe normally while wearing it). The rate at which your body burns calories will be determined by measuring the rate of oxygen that you consume and the carbon dioxide that you produce. This particular test is optional.

Another optional test may take place during days 24-27 of each phase; and you may participate in it. On day 24, you may be asked to consume a small quantity of "labelled" cholesterol mixed in a teaspoon of margarine on half an English muffin. This cholesterol is nearly identical to the regular cholesterol, which the body absorbs from foods or produces endogenously, but can be distinguished and traced in blood. A fasting blood sample (~1 tablespoon) will be collected before the intake of this meal and in the following 3 mornings. On day 27, after blood draw, you will also consume 3 tablespoons of tagged water. This test does not pose any health risk, and will allow us to find out how much dietary cholesterol your body absorbs and how much cholesterol it produces. Fecal samples may be collected during this test.

We recommend that you do not make blood donation during, and for 2 months following participation in, this study. Dietary counseling will be provided to you throughout the study period. Please make sure to report any changes in your health status at any point to the study coordinators.



Risks and Discomforts

As with any clinical trial, there may be as yet unknown or unforeseen risks of taking part. Some known risks, although rare, are associated with placing a needle into a vein. These include the possibility of infection, perforation or penetration of the needle through the vein, and bleeding, pain, or bruising at the site. The technique that will be used to assess the health of blood vessels may cause slight numbness on the forearm, as an effect of a pressure with an armband. This discomfort, if any, is limited to the period of pressure (a few minutes) only. The radiation from DXA test is very low dosage (equivalent to ~1 day of background radiation), and is 1000 times less than the limit for trivial exposure. In case you feel any

Initials of Subject: _____

discomfort during the trial a physician, Dr. Kesselman, will be available to contact at any time. Dr. Kesselman can be reached at (204) 954-4486.

Benefits

You may not benefit from participation in this research; however, the study should contribute to a better understanding of the effect of dairy consumption on human health. It is anticipated that dairy intake will result in positive effects on the inflammatory profile of, and other health-related markers in, the body. In addition, you will receive your results when they become available.

Costs

All clinic and professional fees, diagnostic and laboratory tests that will be performed as part of this study are provided at no cost to you. There will be no cost for the study diet that you will receive. The study cost and honorariums will be covered by Dairy Farmers of Canada, the study sponsor.

Payment for Participation

You will receive up to a maximum of $200 at completion of this study for your time and inconvenience of the study schedule. This amount will be divided into 2 equal portions; 1 portion given after each phase. You will also receive a maximum of $100 upon completing the Study's two optional tests. If you withdraw early from the study, you will receive an appropriate pro-rated fraction of this amount.

Alternatives

You do not have to participate in this study. The study coordinators, physician and principal investigator will answer any questions you have about the experimental group of this study. You should be aware that medications exist as an alternative to treatment of lowering blood inflammatory markers levels.

Confidentiality

Medical records that contain your identity will be treated as confidential in accordance with the Personal Health Information Act of Manitoba. The RCFFN staff involved with your care may review/copy medical information that may reveal your identity. With your permission, the study doctor will also write to your Family Doctor to tell him/her that you are taking part in a study or to obtain further medical information. The Biomedical Research Ethics Board at the University of Manitoba may also review your research-related records for quality assurance purposes. If the results of the trial are published, your identity will remain confidential. Personal information such as your name, address, telephone number and/or any other identifying information will not leave the RCFFN.

You will be assigned a subject code. The coding system of the study for subject identification will be the initials of each subject followed by a three-digit number. The three-digit number will be based on chronological order of subject selection. The identification codes corresponding to the study subjects will be on the written documents which will only be available to the RCFFN staff.

Study samples will be stored in the freezer at the RCFFN. Only the study coordinators and the principal investigator will have access to the samples. Your samples will not be used for any additional analyses nor stored for any longer than 2 years, nor shared with any other group, other than is indicated in the protocol, without your specific consent.

Initials of Subject: _____

Impact of Dairy Consumption on Inflammation: A Clinical Study

Voluntary Participation/Withdrawal from the Study

Your decision to take part in this study is absolutely voluntary. You may refuse to participate or you may withdraw from the study at any time. Your decision to not participate or to withdraw from the study will not affect your other medical care. Your participation in this study may be terminated without your consent by the study coordinators, physician or principal investigator. The study staff will withdraw you if he/she feels that participation is no longer in your best interest, or if you fail to follow the directions of the study staff.

If you Choose to Participate, you will Agree to Cooperate Fully with the Study Visit Schedule, and will Follow the Study Staff's Instructions

We will tell you about new information that may affect your health, welfare, or willingness to stay in this study. Should you wish to withdraw your participation from the study, you must inform the study coordinators so that your file can be officially close.

Medical Care for Injury Related to the Study

In the event of an injury that occurs to you as a direct result of participating in this study, or undergoing study procedures you should immediately notify the study physician, Dr. Kesselman at (204) 954-4486 or go to your nearest emergency room to receive necessary medical treatment. You are neither waiving any of your legal rights by signing this consent form nor releasing the investigator or the sponsor from their legal and professional responsibilities. If any health abnormalities are identified in the clinical tests conducted during this experiment, Dr. Kesselman will be contacted, who will inform you of the results.

Questions

You are free to ask any questions that you may have about your treatment and your rights as a research subject. If any questions come up during or after the study or if you have a research-related injury, contact the study doctor and the study staff.

Investigator:	Dr. Peter Jones	Tel No.:	204-474-8883
Study Physician:	Dr. Edward Kesselman	Tel No.:	204-954-4486

For questions about your rights as a research subject, you may contact the Biomedical Research Ethics Board, University of Manitoba at 204-789-3389. Do not sign this consent form unless you have a chance to ask questions and have received satisfactory answers to all of your questions.

Initials of Subject: _____

Impact of Dairy Consumption on Inflammation: A Clinical Study

Consent

For Study Subject: I agree to allow the study physician to inform my family doctor that I am participating in this study or to obtain information regarding my medical history.

Yes ☐ No ☐

1. I have read and understood this Information and Consent Form, and I freely and voluntarily agree to take part in the research study described above.
2. I understand that I will be given a copy of the signed and dated Information and Consent Form. I have received an explanation of the purpose, duration, and procedures of the study, and the potential risks and benefits that I might expect. I was given sufficient time and opportunity to ask questions and to reflect back my understanding of the study to study personnel. My questions were answered to my satisfaction.
3. I agree to cooperate fully with the study doctor and will tell him if I experience any side effects, symptoms, or changes in my health.
4. I am free to withdraw from the study at any time, for any reason, and without prejudice to my future medical treatment.
5. I have been assured that my name, address, and telephone number will be kept confidential to the extent permitted by applicable laws and/or regulations.
6. I agree to be contacted by the research team to consider participating in future studies.
7. By signing and dating this document, I am aware that none of my legal rights are being waived.

Signature:_____ Date/Time: _____

Printed name of above:_____

_____

For Study Coordinator: I confirm that I have fully explained the details of this research study to the subject whose name and signature appears above. I confirm that I believe that the subject has understood and has knowingly given their consent to participate by his/her personally dated signature.

Signature:_____ Date/Time:_____

Printed name of above:_____ Study role:_____

ALL SIGNATORIES MUST DATE THEIR OWN SIGNATURE

Initials of Subject: _____

Appendix II

Subject Consent Form Corresponding to Genetics Studies Described in Chapters V and VI

UNIVERSITY OF MANITOBA

196 Innovation Drive, Smartpark
University of Manitoba, Winnipeg, MB R3T 2N2
Tel: 204.298.5483 Fax: 204.474.7552

Richardson Centre for Functional Foods and Nutraceuticals

RESEARCH SUBJECT INFORMATION AND CONSENT FORM

ADDITIONAL RESEARCH SUBJECT INFORMATION AND CONSENT FORM FOR GENETIC ANALYSIS

Title of Study: **The Impact of Dairy Consumption on Inflammation: A Clinical Study**

Principal Investigator: **Peter Jones, PhD**
Richardson Centre for Functional Foods and Nutraceuticals (RCFFN)
University of Manitoba
196 Innovation Drive, Smartpark
Winnipeg, Manitoba R3T2N2
Phone: (204) 474-8883

Sponsor: **Dairy Farmers of Canada**
21 Florence Street
Ottawa, ON K2P 0W6
Phone: (613) 236-9997, fax: (613) 236-0905
Email: info.policy@dfc-plc.ca

You are being asked to participate in a research study. Please take your time to review this Information and Consent Form and discuss any questions you may have with the study staff. You may take your time to make your decision about participating in this clinical trial and you may discuss it with your regular doctor, friends and family. This consent form may contain words that you do not understand. Please ask the study doctor or study staff to explain any words or information that you do not clearly understand.

NATURE AND DURATION OF PROCEDURE

From the blood drawn during the clinical study entitled "The Impact of Dairy Consumption on Inflammation: A Clinical Study", we would like to extract DNA and perform genetic analyses using a laboratory technique that augments and recognizes specific genes. This will allow us to determine why some people show enhanced health effects, such as a better decrease in cholesterol levels, than others when consuming low-fat dairy products. DNA is a molecule found in the cells of your body that is organized into genes that contain all of the information needed to make the proteins, which perform specific biological functions in your body

CONFIDENTIALITY AND SAFEKEEPING OF DNA SAMPLES

All of the information obtained about you and the results of the research will be treated confidentially. We will protect your confidentiality by assigning your DNA sample a specific code. This code will link you to your DNA sample and can only be decoded by the principal researcher or an individual authorized by the latter. Samples of your DNA will be kept at the Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba, under the supervision of Dr. Peter Jones for a 2-year period following the end of the

Initials of Subject: _____

research project. After this time, all samples will be destroyed. Your DNA samples will only be used for the purpose of this research project.

Your participation and the results of the research will not appear in your medical record. Although the results of this study may be published or communicated in other ways, it will be impossible to identify you. Unless you have provided specific authorization or where the law permits or a court order has been obtained, your personal results will not be made available to third parties such as employers, government organizations, insurance companies, or educational institutions. This also applies to your spouse, other members of your family and your physician. However, for the purposes of ensuring the proper management of research, it is possible that a member of an ethics committee, a Health Canada representative, or a representative from the Richardson Center for Functional Foods and Nutraceuticals may consult your research data and record. You can communicate with the research team to obtain information on the general progress or the results of the research project. Project updates will be mailed at the end of the project. However, we will not communicate any individual results to you.

POTENTIAL RISKS AND/OR BENEFITS

As the DNA will be extracted from blood samples that have already been taken, there is no additional invasive procedures to undergo and no physical risks to you. While there may be no direct benefits to you for taking part in these additional analyses, we hope that the results we obtain will provide us with novel information on genetic characteristics of people in which the intake of dairy products provided better health impacts during the study time, particularly with regard to cholesterol levels.

SIGNATURE OF PARTICIPANT

The content of the procedure and procedures associated with it have been fully explained to me, and all experimental procedures have been identified. I have had the opportunity to ask questions concerning any and all aspects of the project and procedures involved, and may continue in the future to ask further questions at any time, as it is my right to do so. I am aware that I may refuse to participate as well as withdraw my consent at any time. I acknowledge that no guarantee or assurance has been given by anyone as to the results to be obtained and that my participation in this study is completely voluntary. Confidentiality of records concerning my involvement in this project will be maintained in an appropriate manner. Samples will neither be utilized for any additional analyses, nor stored for any prolonged period, or shared with any other group other than is indicated in the protocol without my specific consent.

I,_____, have read the above description. I have been made aware of all the procedures, advantages and disadvantages of the study, which have been explained to me. By signing this consent form, I have not waived any of the legal rights that I have as a participant in a research study.

_____ _____

Signature of Subject Date

Initials of Subject: _____

Subject Screening Form

UNIVERSITY OF MANITOBA

Eligibility Chart

DATE OF OBSERVATION			INITIALS OF SUBJET		# SUBJECT(CODIFICATION)	
DAY	MONTH	YEAR	NAME	FAMILY NAME	PLI	

Selection Criteria	**YES**	**NO**
1. Aged between 18 et 70 years. Age: _____	❑	❑
2. Men ❑	❑	❑
Pre-menopausal women with menstrual regular cycle ❑ (contraceptive agents ok)		
Post-menopausal women ❑ (who have not initiated hormone replacement therapy within 6 months)		
3. Dairy intake < 2 servings per day: _____ servings		
4. Waist circumference : _____ cm	❑	❑
Men : ≥94 cm		
Women : ≥ 80 cm		
5. Stable weigh since 6 month (+/- 5 lbs)	❑	❑
6.CRP > 2.0 and < 10.0 mg/l:_____ mg/l (S1 and S2)	❑	❑
7. Non-smoking	❑	❑
8. BMI< 35 kg/m^2: BMI: _____	❑	❑
9. Without medication for lipids and high blood pressure	❑	❑
10. Without anti-inflammatory		
11. Without clinical use of vitamin D and Ca supplements	❑	❑
12. Without history of CVD, diabetes, monogenic dyslipidemia, endocrine disorders, gastrointestinal disorder,	❑	❑
13. Without particular food habits (eg : vegetarianism)	❑	❑
14. Without aversion/intolerance/allergy to dairy	❑	❑
15. Blood pressure < 140/90 mmHg	❑	❑

Eligibility	**YES**	**NO**
Subject eligible to participate	❑	❑

UNIVERSITY OF MANITOBA

DATE OF OBSERVATION			INITIALS OF SUBJECT		# SUBJECT(CODIFICATION)	
DAY	MONTH	YEAR	NAME	FAMILY NAME	PLI	

General Information

Family Name:_____ Name: _____

Gender: ❑ W ❑ M

Date of birth (D/M/Y): ___/___/_____ Age: _____

Coordinates:

Address: _____ Postal Code: _____

Telephone: _____ *(home)* _____ *(office)*

_____ *(cell phone)* _____ *(other)*

Email: _____

Coordinates of another person to join (in case):

Family Name: _____ Name: _____

Relationship with the subject: _____ Telephone: _____

Email : _____

Interest and motivation:

What is your interest in participating in this research project?

_____

How did you get information about this research project?

_____

Availability for the meetings at the research center?

Availability in the morning: ❑ Yes ❑ No *Precision:* _____

Availability in the afternoon: ❑ Yes ❑ No *Precision:* _____

Availability in the evening: ❑ Yes ❑ No *Precision:* _____

Preferences:

❑Monday ❑Tuesday ❑Wednesday ❑Thursday ❑Friday ❑Saturday* ❑Sunday*

** If there is no other alternative*

Are you planning a trip or travel outside Manitoba over the next few weeks or months?

❑ Yes ❑ No

If yes, precise: _____

COMMENTS

_____

_____

UNIVERSITY OF MANITOBA

DATE OF OBSERVATION DAY	MONTH	YEAR	INITIALS OF SUBJECT NAME	FAMILY NAME	# SUBJECT(CODIFICATION) PLI	

Medical Questionnaire/Life Habits
SCREENING 1

Date of birth: _____ **Age:** _____

BLOOD SAMPLES: CHECKLIST

SAMPLINGS:	☐ LEFT ARM	☐ RIGHT ARM
BLOOD SAMPLING:	☐ **Yes**	☐ **NO**
EXTRA BLOOD SAMPLE (Keep frozen)	☐ **Yes**	☐ **NO**
HEMOLYSIS:	☐ **Low**	☐ **Strong**

Comments: _____

Nurse's signature: _____

ANTHROPOMETRIC MEASURMENTS

Height (m): _____ **Body weight (kg):** _____ **BMI (kg/m^2):**_____

Waist girth (cm): _____ **Hip girth (cm):** _____ **Ratio:** _____

Completed by: _____

UNIVERSITY OF MANITOBA

DATE OF OBSERVATION DAY	MONTH	YEAR	INITIALS OF SUBJECT NAME	FAMILY NAME	# SUBJECT(CODIFICATION) PLI	

Medical Questionnaire/Life Habits
SCREENING 2

Date of birth: _____ **Age:** _____

BLOOD PRESSURE AT REST (AFTER 10 MINUTES OF REST)

DOMINANT ARM (Pressure): ☐ **Left** ☐ **Right**

TYPE OF DEVICE: ☐ **Mercury** ☐ **Automatic**

10 min systolic BP (mmHg) = _____ diastolique BP (mmHg) = _____
13 min systolic BP (mmHg) = _____ diastolique BP (mmHg) = _____
16 min systolic BP (mmHg) = _____ diastolique BP (mmHg) = _____
Avg. Systolic BP (mmHg) = _____ **Ave. diastolic BP (mmHg) =** _____
Heart rate/min = (HR) 1:_____ HR 2:_____ HR 3:_____

Completed by: _____

BLOOD SAMPLES: CHECKLIST

SAMPLING:	☐ LEFT ARM	☐ RIGHT ARM
BLOOD SAMPLING:	☐ **Yes**	☐ **NO**
EXTRA BLOOD SAMPLE (Keep frozen)	☐ **Yes**	☐ **NO**
HEMOLYSIS:	☐ **Low**	☐ **Strong**

Comments: _____

Nurse's signature: _____

ANTHROPOMETRIC MEASURMENTS

Body weight (kg): _____

MEDICAL HISTORY

Family history	**YES**	**NO**	**Don't Know**	**If Yes, specify** F=father, M=mother, S=siblings, GP=grand parents, U=unknown (ex : adopted)
Type 1 Diabetes	❑	❑	❑	_____
Type 2 Diabetes	❑	❑	❑	_____
Cardiovascular diseases (Class 1)**	❑	❑	❑	_____
Cardiovascular diseases (Class 2)***	❑	❑	❑	_____
Hypertension	❑	❑	❑	_____
Dyslipidemia	❑	❑	❑	_____
Endocrine disorders	❑	❑	❑	_____
Cancers	❑	❑	❑	_____
Others	❑	❑	❑	_____

Personal history	**YES**	**NO**	**Don't Know**	**If Yes, specify**	**Age (dx) ***
Type 1 Diabetes	❑	❑	❑	_____	_____
Type 2 Diabetes	❑	❑	❑	_____	_____
Cardiovascular diseases (Class 1)**	❑	❑	❑	_____	_____
Cardiovascular diseases (Class 2)***	❑	❑	❑	_____	_____
Hypertension	❑	❑	❑	_____	_____
Dyslipidemia	❑	❑	❑	_____	_____
Endocrine disorders	❑	❑	❑	_____	_____
Gastrointestinal disorders	❑	❑	❑	_____	_____
Hepatic diseases	❑	❑	❑	_____	_____
Kidney diseases	❑	❑	❑	_____	_____
Cancers	❑	❑	❑	_____	_____
Surgeries (past or future)	❑	❑	❑	_____	_____
Others	❑	❑	❑	_____	_____

* *Age at diagnosis*
** *Class 1: infarct, Ischemic heart disease, angina, "bypass"…*
*** *Class 2: stroke, aneurysm, thrombophlebitis, cerebral hemorrhage, peripheral vascular disease …*

MEDICATION Yes ❑ No ❑

Name of drug	Dose	Freq.	Indication	Start	Stop	*
				___/___/____	___/___/___	❑
				___/___/____	___/___/___	❑
				___/___/____	___/___/___	❑
				___/___/____	___/___/___	❑
				___/___/____	___/___/___	❑
				___/___/____	___/___/___	❑

* *Check this box if the participant has not stopped using the drug.*

If you are using cholesterol lowering medication, would you be willing to stop taking it at least 6 weeks before the beginning of the study, with the authorization from your doctor?
Yes ❑ No ❑

Drug Allergies: Yes ❑ No ❑

If yes, specify: _____

Natural health products

Do you consume dietary supplements, vitamins and / or minerals, homeopathic remedies and other natural products (probiotics, medicinal plants, omega-3 ...):
Yes ❑ No ❑

Name of product	Dose	Freq.	Indication	Start	Stop	*
				___/___/____	___/___/___	❑
				___/___/____	___/___/___	❑
				___/___/____	___/___/___	❑
				___/___/____	___/___/___	❑
				___/___/____	___/___/___	❑
				___/___/____	___/___/___	❑
				___/___/____	___/___/___	❑
				___/___/____	___/___/___	❑

* *Check this box if the participant has not stopped using the natural health product*

Would you be willing to stop taking these supplements at least 6 weeks before the beginning of the study?
Yes ❑ No ❑

WOMEN (PREGNACY-GYNECOLOGY)

Are you postmenopausal? Yes ❑ No ❑

Premenopausal women

Are you currently taking oral contraceptives or other forms of hormonal contraception?
Yes ❑ No ❑

Do you have a regular menstrual cycle? Yes ❑ No ❑ *Not applicable* ❑

What is the average length of your menstrual cycle? _____

Date of your last menstruations: _____

Are you pregnant? Yes ❑ No ❑

Are you planning a pregnancy soon? Yes ❑ No ❑
If yes, how long? _____

Postmenopausal women

Age at menopause: _____ Yrs *Don't know* ❑

Type of menopause:
Natural ❑ *Hysterectomy* ❑ *Hysterectomy + partial oophorectomy (1 ovary)* ❑
Hysterectomy + total oophorectomy (2 ovaries) ❑ *Tubal ligation* ❑
Caused by chemotherapy or other treatment ❑ Don't know ❑

Are you currently taking hormone replacement? Yes ❑ No ❑
If yes, start date: ___/___/___
If yes, type of hormones: _____

Have you ever taken hormone replacement in the past? Yes ❑ No ❑
If yes, start date: ___/___/___
If yes, type of hormones: _____

LIFE HABITS

Tobacco	**Yes ❑ No ❑**	Date of arrest (if any): _____ If yes, how often: _____
Street drug	**Yes ❑ No ❑**	Date of arrest (if any): _____ If yes, how often: _____
Alcohol	**Yes ❑ No ❑**	Type(s) of alcohol: _____ Quantity consumed: _____ How often: _____

NUTRITION

	YES	NO	Don't know	If yes, specify
Allergies:				
Foods	❑	❑	❑	_____
Others	❑	❑	❑	_____
Intolerances	❑	❑	❑	_____
Special dietary habits (E.g. vegetarianism)	❑	❑		_____
Consumption of:				
Soft drinks	❑	❑	❑	_____
Energy drinks	❑	❑	❑	_____
Coffee	❑	❑	❑	_____
Tea	❑	❑	❑	_____

Number of meal /day: _____

PHYSICAL ACTIVITY

How often per week do you practice physical activity for a period of more than 30 minutes?

_____

What type of physical activity do you practice?

_____

_____

SCHEDULE

_____

_____

COMMENTS

_____

_____

UNIVERSITY OF MANITOBA

DATE			SUBJECT INITIALS		# SUBJECT (CODIFICATION)	
DAY	MONTH	YEAR	NAME	FAMILY NAME	PLI	

Ethnicity Questionnaire

Please check off the appropriate square(s).

❑$_1$ Caucasian

❑$_2$ Aboriginal

❑$_3$ Black (e.g. African, Haitian, Jamaican, Somali)

❑$_4$ Hispanic (e.g. South American, Latin American)

❑$_5$ Arab/West Asian (e.g. Armenian, Egyptian, Iranian, Lebanese, Morrocan)

❑$_6$ Chinese

❑$_7$ Japanese

❑$_8$ Korean

❑$_9$ South East Asian (e.g. Cambodian, Indonesian, Vietnamese)

❑$_{10}$ South Asian (e.g. East Indian, Pakistani, Punjabi, Sri Lankan)

❑$_{11}$ Filipino

❑$_{12}$ Other: _____

Dietary Recommendations

UNIVERSITY OF MANITOBA

PLI project – Recommendations

Dietary recommendations during the study

- Respect the **eatwell plate**:

  - Half of the plate should be reserved for vegetables.
  - Quarter of the plate should be attributed to the grain products (the size of a fist).
  - The other quarter is for the meat, fish and alternatives (the size of the palm of the hand)
  - Add a fruit for dessert

- **Limit** your intake of saturated fats and **avoid *trans* fats**:
  - Saturated fats are found mostly in animal products like butter, cheese, red meat and processed meats but also in some vegetable products like coconut oil or palm oil.
  - *Trans* fats are found in many transformed commercial products such as pastries, cookies, chips, fried foods, margarine made from hydrogenated or partially hydrogenated oils and shortening or lard.

- **Limit** your intake of **dietary cholesterol**. Cholesterol is mainly found in foods of animal origin. The main sources of cholesterol are eggs, butter, meat offal (liver and heart), sausages, deli meat and meat.

- ✓ A medium size whole fruit or vegetable
- ✓ 125 ml of 100% pure juice
- ✓ 125 ml of fresh, frozen or canned vegetables or fruits
- ✓ 125 ml of cooked leafy vegetables
- ✓ A 250 ml bowl of lettuce
- ✓ 250 ml of raw leafy vegetables

- Choose **whole** grain products:
  Prefer whole wheat bread, cereals high in dietary fiber and low in refined sugar, brown rice, whole wheat pasta etc. In your recipes, substitute the white flour for whole wheat flour.

- **Limit** your **sodium intake**. Most part of the sodium we eat comes from **processed foods** like crackers, cereals, soups, vegetable juices, salad dressings, frozen dinners, chips, cheeses, deli meats and canned foods. Cook as much as possible at home and **choose products with reduced, low or no sodium.**

Specifications for the different diets

- During the diet with dairy products, you should eat **only** dairy products that will be provided. You can't consume other sources of dairy products like cream cheese or other processed cheese and kefir (fermented milk). Butter, ice cream and sour cream are not considered like dairy products.

- During the diet without dairy products, it's important to **don't consume any dairy products**. Beware of **hidden sources** of dairy products like the milk in various recipes (pancakes, omelette, quiche, oatmeal), gratin dishes like lasagna, pizza, milk or cream in coffee or tea, cream soup etc.

- During the run-in period (2 weeks before the start of the study) and the wash-out period (between the two diets), you should limit your consumption of dairy products to 2 servings daily. Prefer dairy products low in fat like milk and yogurt with less than 2% fat or cheese with less than 20% fat.

A serving of dairy product is:

- ✓ 250 ml of milk
- ✓ 175 g of yogurt
- ✓ 50 g of cheese

- You **can't consume any soy products** during the 12-week study (soy beverages, tofu, soybeans, soya dessert (pudding) or cooking or coffee cream). **Substitute of milk** (cereal based drinks or almond beverages) can be consumed **except if they are fortified with calcium**.

Caffeinated beverages

- **Limit to 2 cups (250 ml) per day** your consumption of caffeinated beverages like coffee, tea, energy drinks and soft drink of cola type.

Alcohol

- Limit to **1 drink per day** you alcohol intake for a maximum of 5 per week.

  One serving of alcohol is:

  - ✓ A normal size bottle of beer (340 ml)
  - ✓ A glass of wine of 150 ml
  - ✓ A glass of liquor of 45 ml

  ***Important, these servings are not cumulative.

Supplements and Natural Health Products

- Stop taking any dietary supplements such as multivitamins or natural health products.

Medication

- You must maintain your drug use stable during the study. **You can't consume ibuprofen** (Advil, Motrin) during the study, **only Tylenol as needed**. If you need to consume any medication, you have first to notify the study coordinator and to note it on the checklist in the space provided.

Physical Activity

- Keep your level of physical activity constant throughout the duration of the study.

Blood sample

- Blood sample only: You must complete a 12-h fast, but you can drink water to facilitate the blood sample. 48 h before blood sampling, you can't drink alcohol and refrain from making intense physical activity (aerobic, spinning, and running).

- Blood sample with an EndoPAT (week 4 and 12): You must complete a **12-h fast**, but you can drink water to facilitate the blood sample. **72 h before** blood sampling, you **can't drink alcohol or caffeinated beverages** such as coffee, tea, energy drinks or soft drinks (cola type) and **refrain from making intense physical activity** (aerobic, spinning, and running). Finally, **12-h before** blood sampling, **you can't take Tylenol**.

Body composition Test (DXA)

- For the body composition test, it will be important **not to wear cloths with metal parts** (buttons, belt). A hospital gown will be available, if needed.

Thank you for your cooperation

Notes: _____

Appendix II

Study Products' Dietary Compositions

DAIRY DIET

Food	Serving size	kcal	Protein (g)	Carbs (g)	Fiber (g)	Lipids (g)	SFA (g)	MUFA (g)	PUFA (g)	Na (mg)	Ca (mg)
1% fat milk	375 ml	162	13	19	0	3.8	1.2	0.58	0.07	170	483
Regular cheddar cheese 34% fat	30 g	120	7	0	0	10	6	2.84	0.28	210	216
Creamy Stirred Yogurt Yoplait 1.5% fat (fruit flavours)	175 g	150	6	26	0	2.5	1.5	0.69	0.07	85	214
Total		**432**	**26**	**45**	**0**	**16.3**	**8.7**	**4.11**	**0.42**	**465**	**913**

Fruit flavors available (650 g): Strawberry, Cherry and sunnyfruit.

CONTROL DIET

Food	Serving size	kcal	Protein (g)	Carbs (g)	Fiber (g)	Lipids (g)	SFA (g)	MUFA (g)	PUFA (g)	Na (mg)	Ca (mg)
Fruit juice	290 ml	133	0.4	31.3	0	0	0	0	0	29	46
Original V8 100% vegetable juice	156 ml (small can)	30	1	7	1	0	0	0	0	300	18
Planters Salted cashews	20 g	125	4	6	1.5	9.5	1.8	5.6	1.61	58	10
Cookies (homemade)*	1 cookie (39 g)	150	2.3	19	0.7	7.6	5.8	1.0	0.3	72	10
Total		**438**	**7.7**	**63.3**	**3.2**	**17.1**	**7.6**	**6.6**	**1.91**	**459**	**84**

*Nutrifiq Software

Study Cookies Recipe

PLI project

Chocolate chips cookies recipe

Ingredients for 1 cookie:

Coconut oil	5,5	g
Brown sugar	5,4	g
Egg, beaten	2,8	g
Water	5	g
Baking soda	0,1	g
Salt	0,1	g
Semi-sweet chocolate chips	4,9	g
All-purpose flour	14,8	g

Ingredients for 25 cookies:

Coconut oil	137,5	g
Brown sugar	135	g
Egg, beaten	70	g
Water	125	g
Baking soda	2,5	g
Salt	2,9	g
Semi-sweet chocolate chips	122,5	g
All-purpose flour	370	g

Ingredients for 50 cookies:

Coconut oil	275	g
Brown sugar	270	g
Egg, beaten	140	g
Water	250	g
Baking soda	5	g
Salt	5,8	g
Semi-sweet chocolate chips	245	g
All-purpose flour	740	g

Ingredients for 100 cookies:

Coconut oil	550	g
Brown sugar	540	g
Egg, beaten	280	g
Water	500	g
Baking soda	10	g
Salt	11,6	g
Semi-sweet chocolate chips	490	g
All-purpose flour	1480	g

Directions:

- Preheat oven to 350°F.
- Cream together the coconut oil and brown sugar. Beat in the eggs.
- Dissolve baking soda in hot water. Add to batter along with salt. Stir in flour and chocolate chips.
- Drop into ungreased pans.
- Bake for about 10 minutes in the preheated oven, or until edges are nicely browned.

Appendix II

Food Journal

UNIVERSITY OF MANITOBA

DAIRY PRODUCTS AND INFLAMMATION

FOOD JOURNAL

Subject number: _____

Reception date: _____

REMINDER

. List everything you eat and drink during 3 days (2 days in the week and 1 day in the week-end).

. This food journal have to represent your normal diet.

. Indicate the day, date, time and the place of the meal.

. List only one food per line. Use as many sheets as you need for each day.

. Note progressively what you eat to not make oversights.

. Provide as much details as possible for each food. (« it's better to indicate more than less »).

. Indicate the quantity by volume (cup, mL, teaspoon, and tablespoon) or in weight (gram or ounce) of everything you eat and drink. Indicate whether the measure was taken before of after cooking and if it includes fat, bone, skin etc.

. When you describe a food, you must indicate:

- The method of cooking (roasted, fried, boiled, grilled, stir-fried, with or without fat ect.)
- The form food (fresh, frozen, canned etc.)

. List the condiments and other accompaniments (ketchup, sauce, salt, oil, butter etc.)

. Indicate the trademark of the food if it's possible. You can also cut the label of the product including the nutrition facts table if available.

. Don't forget to write everything you eat including snacks, gum, candy, beverages and medication

. When you eat a mixed meal (Ex : Shepherd's Pie) or any other recipe, please write the recipe with all the ingredients and their quantity. Note the number of servings of the recipe and the amount consumed.

. If you eat at a restaurant, please indicate the name of the restaurant, food eaten and your best estimation of the serving size consumed.

Thank you for your participation at this research project.
Your collaboration is very appreciated.

FOOD JOURNAL

SUBJECT #:

DAY: MONDAY DATE: 30 AOÛT 1999

EXAMPLE

Time	Place	Quantity (gram/vol.)	Description of the food or drink consumed (one food per line)	Trademark	Don't write in this column
		1	Bread and margarine		
		1 bowl	Vegetable soup		
		A little	Salad with dressing		
		1 piece	Shepherd's pie		
		1 glass	Milk		
		2	Cookies		
18 :15	Home	1 slice = 28 g	Whole wheat bread	Bon Matin	
		1 teaspoon	Original margarine	Becel	
		250 mL	Vegetable soup canned, with pasta, diluted with water	Campbell	
		50 g	Iceberg lettuce		
		20 g	Grated carrots		
		20 mL	Regular French dressing	Kraft	
		1 serving	Shepherd's pie ➔ see recipe		
		250 mL	Milk 1% m.f.	Lactantia	
		2 cookies. = 40g	Vanilla sandwich cookies	David	
20 :00	Home	250 mL	Coffee		
		2 teaspoon	Sugar		

EXAMPLE NOT TO FOLLOW

EXAMPLE TO FOLLOW

FOOD JOURNAL

SUBJECT #:

Day: _____ *Date:* _____

Time	Place	Quantity (gram./vol.)	Description of the food or drink consumed (one food per line)	Trademark	Did you mention these details?
					✓ **Juice?** Real juice, sweet beverages, powder, cocktail ✓ **Bread?** White, wheat, cereals ✓ **Topping?** Amount of : butter, peanut butter, jam, cheese ✓ **Muffin? 1) Size** : small, medium, large **2) Type** : homemade or commercial **3) Sort** ✓ **Egg? 1) Sort**: Fried, boiled, scrambled, poached 2) fat added for cooking? amount ✓ **% fat dairy products?** milk, cheese, yogurt ✓ **Sugar, cream or syrup added?** In coffee, cereal, on pancakes… ✓ **Soup?** homemade, canned, bagged ✓ **Salad? 1) Components** : amount of vegetables, cheese, croutons, seeds and nuts…**2) Dressing** : a)type : creamy, Italian… b) amount : ml, teaspoon, tablespoon, gram ✓ **Sandwich? 1) Bread**: White, wheat, cereals **2) Condiments**: amount of mayonnaise, butter, mustard… ✓ **Meat, fish?** Cooking: oven, pressure cooker, Teflon pan, on the grill… ✓ **Poultry?** Did you consume the skin? ✓ **Fat added for cooking?** Amount of butter, margarine or oil ✓ **Dessert? 1) Piece size** : in centimeters, in inches, or in fraction **2) Topping** : frosting, cream, ice cream, syrup, sauce … ✓ **Snacks? 1) Type** : chips, pretzels, salt crackers…**2) Amount** : in grams/unit

FOOD JOURNAL

SUBJECT #:

Day: _____ *Date :* _____

Time	Place	Quantity (gram./vol.)	Description of the food or drink consumed (one food per line)	Trademark	Did you mention these details?
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FOOD JOURNAL

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FOOD JOURNAL

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FOOD JOURNAL

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Subject #:

Recipe : **Example** *Shepherd's Pie* **Recipe's yield: 4 servings**		**Recipe :** **Recipe's yield: _____**	
1 pound	*Mid lean ground beef*		
2 large	*Minced onions*		
½ teaspoon	*Savory*		
2 mL	*Salt and pepper*		
14 oz.	*Canned, cream corn*		
4 cups ½ cup	*Baked potatoes, mashed*		
½ cup	*2% fat Milk*		
Recipe : **Recipe's yield: _____**		**Recipe :** **Recipe's yield: _____**	

SUBJECT # :

Recipe :	Recipe's yield: _____	Recipe :	Recipe's yield: _____

Recipe :	Recipe's yield: _____	Recipe :	Recipe's yield: _____

Physical Activity Journal

UNIVERSITY OF MANITOBA

Subject #: _____

How to complete your 3-day physical activity journal

The physical activity journal is used to assess your usual level of activity. The three days must be chosen the same as your diet records: two days of the week and one day of the weekend. You have a sheet to complete for each day. Here's an example:

	Minutes			
Hour	**0-15**	**16-30**	**31-45**	**46-60**
0 A.M.	2	2	2	1
1	1	1	1	1
2	1	1	1	1
3	1	1	1	1
4	1	1	1	1
5	1	1	1	1
6	1	1	1	1
7	1	1	1	1
8	1	1	1	1
9	3	4	3	4
10	5	5	5	5
11	5	5	5	5
12 P.M.	5	5	5	2
13	2	2	5	5
14	5	5	5	5
15	5	5	2	2
16	2	2	2	2
17	2	2	4	2
18	2	2	4	4
19	4	4	4	4
20	2	2	2	2
21	8	8	8	4
22	4	4	2	2
23	2	2	1	1

Subject #: _____

3-day physical activity journal

DAY 1

Date (d/m/y) : _____

INSTRUCTIONS

Each rectangle to the right of the column of hours matches with a period of 15 minutes. Each hour is divided into 4 periods of 15 minutes. From the list of activities given in the last 2 pages, enter the code for the activity you have performed during each period of 15 minutes. If an activity is practiced for a long period (eg sleep), you can make a continuous horizontal line in the boxes below, until you change your activity.

Hour	Minutes			
	0-15	16-30	31-45	46-60
0 A.M.				
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12 P.M.				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				

Subject #: _____

3-day physical activity journal

DAY 2

Date (d/m/y) : _____

INSTRUCTIONS

Each rectangle to the right of the column of hours matches with a period of 15 minutes. Each hour is divided into 4 periods of 15 minutes. From the list of activities given in the last 2 pages, enter the code for the activity you have performed during each period of 15 minutes. If an activity is practiced for a long period (eg sleep), you can make a continuous horizontal line in the boxes below, until you change your activity.

	Minutes			
Hour	**0-15**	**16-30**	**31-45**	**46-60**
0 A.M.				
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12 P.M.				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				

Subject #: _____

3-day physical activity journal

DAY 3

Date (d/m/y) : _____

INSTRUCTIONS

Each rectangle to the right of the column of hours matches with a period of 15 minutes. Each hour is divided into 4 periods of 15 minutes. From the list of activities given in the last 2 pages, enter the code for the activity you have performed during each period of 15 minutes. If an activity is practiced for a long period (eg sleep), you can make a continuous horizontal line in the boxes below, until you change your activity.

	Minutes			
Hour	**0-15**	**16-30**	**31-45**	**46-60**
0 A.M.				
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12 P.M.				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				

Subject #: _____

Categories	Examples of activities for each category		
1	**Lying :** - Sleeping	- Bed rest	
2	**Sitting :** - Listening in class - Listening the radio or television - Writing, working on computer	- Bathing - Eating - Reading	- Car ride (passenger) - Driving a car
3	**Very light activities:** - To wash, take a shower - Combing hair, shaving, put make-up - Make the bed - Get dressed	- Cooking - Ironing clothes - Dishwashing - To dust	- Driving a truck, a bus - Driving a motorcycle - Billiards , croquet - Supervisor of machinery
4	**Light activities:** - Walking slowly - Play music - Bowling	- Throwing and catching (ball, Frisbee) - Sewing - Nursing	
5	**Light manual work :** - Moderate walking (go to school, to work, etc.) - Housework (sweeping, cleaning windows, etc.) - To garden, mowing - Archery	- Snowmobiling - Electrician, plumber - Carpenter, painter - Mechanician - Baker	
6	**Sports or light leisure activities (recreation) :** - Sailing - Kayaking * - Golf - Baseball (except the pitcher) - Bicycle (ride) *	- Aerobics - Ping-Pong - Horseback riding * - Fitness - Dancing (couple, line)	- Volleyball *
7	**Moderate manual work, sports or leisure activities :** - Manoeuvring (building industry, working mine) - Shoveling snow - Loading bags or crates - Work of planting or forest * (chainsaw or handling of logs)		- Alpine skiing - Baseball (pitcher) - Badminton * - Tennis *
8	**Intense manual work, sports or leisure activities (non-competitive) :** - Remove tree branches - Sawing by hand - Brisk walking * - Cycling (rapid) * - Ice skating, rollerblading *	- Aerobic, Step * - Swimming * - Rowing * - Snowshoeing * - Jogging	- Hiking * - Judo, karate, boxing *
9	**Extreme manual work, sports or leisure activities (competitive) :** - Felling trees with axes * - Climbing * - Soccer * - Handball * - Squash *	- Basketball * - Racquetball * - Skiing * - Running - Hockey *	- Football, ultimate Frisbee *

* For the categories 6 to 9, refer to table below before classifying activities annotated with an asterisk (*). You can decide to classify the activity in a different category than that indicated above.

Categories	
6	Activities performed at a speed of walking, mild and recreationally
7	Moderate manual work, activities or sports
8	Intense activities for a non competitive purpose
9	Extreme activities or for a purpose of competition

3-day physical activity diary - Categories

Medical Questionnaires

UNIVERSITY OF MANITOBA

DATE OF OBSERVATION DAY	MONTH	YEAR	INITIALS OF SUBJECT NAME	FAMILY NAME	# SUBJECT(CODIFICATION) PLI	

Medical Questionnaire/Life Habits
Week 0 ☐ Week 4 ☐ Week 8 ☐ Week 12 ☐

BLOOD PRESSURE AT REST (after 10 minutes of rest)

DOMINANT ARM (Pressure): ☐ Left ☐ Right

TYPE OF DEVICE: ☐ **Mercury** ☐ **Automatic**

10 min systolic BP (mmHg) = _____ diastolic BP (mmHg) = _____
13 min systolic BP (mmHg) = _____ diastolic BP (mmHg) = _____
16 min systolic BP (mmHg) = _____ diastolic BP (mmHg) = _____
Avg. Systolic BP (mmHg) = _____ **Ave. diastolic BP (mmHg)** =_____
Heart rate/min = (HR) 1:_____ HR 2:_____ HR 3:_____

Completed by: _____

BLOOD SAMPLES: CHECKLIST

SAMPLINGS:	☐ LEFT ARM	☐ RIGHT ARM
BLOOD SAMPLING:	☐ **Yes**	☐ **NO**
EXTRA BLOOD SAMPLE (Keep frozen)	☐ **Yes**	☐ **NO**
HEMOLYSIS:	☐ **Low**	☐ **Strong**

Comments: _____

Nurse's signature: _____

ANTHROPOMETRIC MEASUREMENTS

Body weight (kg): _____

Waist girth (cm): _____ _____ _____

Hip girth (cm): _____ _____ _____

Completed by: _____

CHANGE IN MÉDICATION **Yes ❑** **No ❑**

Name of drug	Dose	Freq.	Indication	Start	Stop
				___/___/____	___/___/___
				___/___/____	___/___/___
				___/___/____	___/___/___
				___/___/____	___/___/___
				___/___/____	___/___/___
				___/___/____	___/___/___

Date of your last menstruations: _____

Comments:

_____
_____
_____
_____
_____

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DATE OF OBSERVATION			INITIALS OF SUBJECT		# SUBJECT(CODIFICATION)	
DAY	MONTH	YEAR	NAME	FAMILY NAME	PLI	

Medical Questionnaire/Life Habits
WEEK 2 ☐ WEEK 10 ☐

ANTHROPOMETRIC MEASUREMENTS

Body weight (kg): _____

Waist girth (cm): _____ _____ _____

Hip girth (cm): _____ _____ _____

Completed by : _____

Comments :

_____
_____
_____
_____
_____

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DATE OF OBSERVATION			INITIALS OF SUJET		# SUBJECT (CODIFICATION)	
DAY	MONTH	YEAR	NAME	FAMILY NAME	PLI	

24-h dietary recall

WEEK 2 ☐ WEEK 10 ☐

Breakfast	Lunch	Dinner
AM snack	**PM snack**	**Evening snack**

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DATE OF OBSERVATION DAY	MONTH	YEAR	INITIALS OF SUBJECT NAME	FAMILY NAME	# SUBJECT(CODIFICATION) PLI	

Medical Questionnaire/Life habits

Week 3 ☐ **Week 11** ☐

BLOOD PRESSURE AT REST (After 10 minutes of rest)

DOMINANT ARM (Pressure): ☐ **Left** ☐ **Right**

TYPE OF DEVICE : ☐ **Mercury** ☐ **Automatic**

10 min systolic BP (mmHg) = _____ diastolic BP (mmHg) = _____

13 min systolic BP (mmHg) = _____ diastolic BP (mmHg) = _____

16 min systolic BP (mmHg) = _____ diastolic BP (mmHg) = _____

Avg. Systolic BP (mmHg) = _____ **Ave. diastolic BP (mmHg) =**_____

Heart rate / min = (HR) 1 :_____ HR 2 :_____ HR 3 :_____

Completed by: _____

BLOOD SAMPLES: CHECKLIST

SAMPLING: ☐ LEFT ARM ☐ RIGHT ARM

BLOOD SAMPLING : ☐ **Yes** ☐ **NO**

EXTRA BLOOD SAMPLE (Keep frozen) ☐ **Yes** ☐ **NO**

HEMOLYSIS : ☐ **Low** ☐ **Strong**

Comments: _____

Nurse's signature: _____

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OBSERVATION DATE DAY	MONTH	YEAR	INITIALS OF SUBJECT NAME	FAMILY NAME	# SUBJECT (CODIFICATION) PLI	

Week 4 ☐ **Week 12** ☐

Bone Densitometry Questionnaire

Full test (whole body, spine, femur)

Age: _____ **Date of birth:** _____ **Height:** _____ **Weight:** _____

Gender: ☐ Woman ☐ Man

Maximum height for whole body (max: 1.88 meters or 6'2 inches)
If weight > 160 kg or 350 lbs, bone density test on the forearm

1. Did you ever taken an examination for osteoporosis at RCFFN?	☐ Yes ☐ No
2. Have you ever had surgery which required the installation of screws, metal plates or dentures? If yes, specify: _____	☐ Yes ☐ No
3. Do you have any piercing?	☐ Yes ☐ No
4. Does a doctor have already diagnosed deformation of the spine (scoliosis)?	☐ Yes ☐ No
5. Do you have a pacemaker?	☐ Yes ☐ No
6. In the last two weeks, have you passed an examination with radiopharmaceuticals products? (barium, nuclear medicine) ***If yes, don't start the test***	☐ Yes ☐ No
7. If you are a woman, are you pregnant? ***If yes, don't start the test***	☐ Yes ☐ Non ☐ N/A
8. Do you take a calcium and/or vitamin D supplement?	☐ Yes ☐ No

Thank you for your collaboration!

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EndoPAT Data Collection Form (PLI)

ENDOPAT VISIT	OBSERVATION DATE			INITIALS OF SUBJECT		# SUBJECT (CODIFICATION)	
Week 4 Week 12	DAY	MONTH	YEAR	NAME	FAMILY NAME	PLI	

DOMINANT ARM: ☐ Left ☐ Right

1. In the last 12 hours, have you consumed any food or drink besides water? Yes No
   If yes, note the time they ate/drank (__ __:__ __ am/pm) and reschedule test.

2. Have you consumed alcohol, alcohol containing products or caffeinated beverages in the last 72 hours?
   Yes No
   If yes, note time (__ __:__ __am/pm) and reschedule test.

3. In the last 72 hours, have you exercised vigorously? Yes No
   If yes, not time they exercised (__ __:__ __am/pm) and reschedule test.

4. *If a Premenopausal Female*: How many days has it been since you last period began? _____
   The participant should be on day 1 through day 7 of menstruation, if not reschedule the test
   ** Please make sure the subject understands that Day 1 is the first day of menstruation**

5. What medications do you take regularly? (List name, dose, frequency)

6. In the last 12 hours, have you taken any medication, prescription or over the counter? Yes No
   Reschedule if participant has taken any antibiotics, meds with a decongestant component (Ephedrine), NSAIDS (Advil, Motrin, Aleve), steroids, or used an inhaler

7. Do you take vitamins or herbal supplements? Yes No

8. Blood Pressure Measurements after a 5 min rest (1 min apart): ___/___ (1st reading)
   ___/___ (2nd reading)
   Enter into EndoPAT Program: ___/___ (Average of 2 readings)

9. Weight: _____(lbs)_____(kg)
10. Height:_____(ft,in)_____(cm)

Room Temp: _____ Gender:_____ Age:_____ Cuff Setpoint:_____

Cm from crease:_____

Begin Rest (yellow)	Begin Baseline (green)	Cuff Inflation	Cuff Deflation	End Study (red)
__/__ am	__/__ am	__/__ am	___ : ___ am	___ : ___ am

Rest = 10min → Baseline = 5min → Occlusion = 5min → Deflation 5min →

RHI: AI: AI@75bpm: HR:

Comments/Problems:

Dietary Checklists

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Date: _____ Week: ____ # subject: _____ Diet: _____

Checklist
DAIRY DIET

Day	Foods	Check if consumed	Coffee, tea, alcohol	Medication
Monday	Milk Yogurt Cheese	( ) ( ) ( )		
Tuesday	Milk Yogurt Cheese	( ) ( ) ( )		
Wednesday	Milk Yogurt Cheese	( ) ( ) ( )		
Thursday	Milk Yogurt Cheese	( ) ( ) ( )		
Friday	Milk Yogurt Cheese	( ) ( ) ( )		
Saturday	Milk Yogurt Cheese	( ) ( ) ( )		
Sunday	Milk Yogurt Cheese	( ) ( ) ( )		

Notes: _____

_____

_____

_____

_____

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Date: _____ Week: _____ # subject: _____ Diet: _____

CHECKLIST
CONTROL DIET

Day	Foods	Check if consumed	Coffee, tea, alcohol	Medication
Monday	Fruit juice Vegetable juice Cookies Cashews	( ) ( ) ( ) ( )		
Tuesday	Fruit juice Vegetable juice Cookies Cashews	( ) ( ) ( ) ( )		
Wednesday	Fruit juice Vegetable juice Cookies Cashews	( ) ( ) ( ) ( )		
Thursday	Fruit juice Vegetable juice Cookies Cashews	( ) ( ) ( ) ( )		
Friday	Fruit juice Vegetable juice Cookies Cashews	( ) ( ) ( ) ( )		
Saturday	Fruit juice Vegetable juice Cookies Cashews	( ) ( ) ( ) ( )		
Sunday	Fruit juice Vegetable juice Cookies Cashews	( ) ( ) ( ) ( )		

Notes: _____

_____

_____

_____

Tests and Documents Checklist

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Tests and documents completed

INITIALS OF SUBJECT NAME	FAMILY NAME	# SUBJECT(CODIFICATION) PLI	

	Given	Completed	Date
Screening 1: - General information form + signature of the screening consent form - Anthropometry - Blood sample		❑ ❑ ❑	
Screening 2: - Blood pressure - Blood sample - Medical questionnaire/Life habits + Ethnicity questionnaire - Consent form signature - 3-day food journal - 3-day physical activity journal - Web-FFQ	 ❑ ❑ ❑	❑ ❑ ❑ ❑ ❑ ❑ ❑	
Week -2 : Dietary recommendations		❑	
Week -1 : Dietary counseling as needed		❑	
Week 0 : Diet 1 _____ - Blood pressure - Anthropometry - Blood sample - Dietary counseling as needed - Foodstuffs - Side effects questionnaires - Checklist	 ❑ ❑	 ❑ ❑ ❑ ❑ ❑ ❑	
Week 1: - Foodstuffs - Checklist	 ❑ ❑	 ❑	
Week 2 : - Anthropometry - Foodstuffs - Checklist - 24h dietary recall - Dietary counseling as needed	 ❑ ❑	 ❑ ❑ ❑	
Week 3 : - Blood pressure - Blood sample - 3-day food journal - 3-day physical activity journal - Web-FFQ - Foodstuffs - Checklist	 ❑ ❑ ❑ ❑ ❑	 ❑ ❑ ❑ ❑ ❑	

	Given	Completed	Date
Week 4: End of diet 1 - Blood pressure - Anthropometry - Blood sample - EndoPAT - Cholesterol kinetics - DXA - Side effects questionnaires - Dietary counseling as needed		❑ ❑ ❑ ❑ ❑ ❑ ❑	
Week 7: Counseling		❑	
Week 8 : Diet 2 : _____ - Blood pressure - Anthropometry - Blood sample - Dietary counseling as needed - Foodstuffs - Side effects questionnaires - Checklist	 ❑ ❑	 ❑ ❑ ❑ ❑ ❑ ❑	
Week 9: - Foodstuffs - Checklist	❑ ❑	 ❑	
Week 10 : - Anthropometry - Foodstuffs - Checklist - 24h dietary recall - Dietary counseling as needed	 ❑ ❑ ❑ ❑	 ❑ ❑ ❑	
Week 11: - Blood pressure - Blood sample - 3-day food journal - 3-day physical activity journal - Web-FFQ - Foodstuffs - Checklist	 ❑ ❑ ❑ ❑ ❑	❑ ❑ ❑ ❑ ❑ ❑	
Week 12 : End of diet 2 - Blood pressure - Anthropometry - Blood sample - EndoPAT - Cholesterol kinetics - DXA - Side effects questionnaires		 ❑ ❑ ❑ ❑ ❑ ❑ ❑	

Side Effects Questionnaires

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DATE OF OBSERVATION			INITIALS OF SUBJECT		# SUBJECT(CODIFICATION)	
DAY	MONTH	YEAR	NAME	FAMILY NAME	PLI	

SIDE EFFECTS QUESTIONNAIRE-FREQUENCY

Week 0 ☐ Week 4 ☐ Week 8 ☐ Week 12 ☐

Indicate if you experienced side effects listed below over the **last four weeks** and if so, how hard was the intensity.

Side effects	**Frequency**			
	Never[0]	Rarely[1]	Sometimes[2]	Often[3]
1. Headache	☐	☐	☐	☐
2. Anxiety	☐	☐	☐	☐
3. Fatigue / exhaustion	☐	☐	☐	☐
4. Lack of energy	☐	☐	☐	☐
5. Tend to become exhausted quickly	☐	☐	☐	☐
6. Decreased appetite	☐	☐	☐	☐
7. Increased appetite	☐	☐	☐	☐
8. Hiccup	☐	☐	☐	☐
9. Nausea	☐	☐	☐	☐
10. Vomiting	☐	☐	☐	☐
11. Indigestion	☐	☐	☐	☐
12. Stomach or abdominal pain	☐	☐	☐	☐
13. Constipation	☐	☐	☐	☐
14. Diarrhea	☐	☐	☐	☐
15. Flatulence	☐	☐	☐	☐
16. Abdominal bloating	☐	☐	☐	☐
17. Palpitations	☐	☐	☐	☐
18. Balance disorders	☐	☐	☐	☐
19. Decreased ability to concentrate	☐	☐	☐	☐
20. Flushing	☐	☐	☐	☐
21. Feeling cold	☐	☐	☐	☐
22. Joint or members pain	☐	☐	☐	☐
23. Numbness, burning or itching (feet/hands)	☐	☐	☐	☐
24. Dark or depressing thoughts	☐	☐	☐	☐
25. Other	☐	☐	☐	☐

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DATE OF OBSERVATION			INITIALS OF SUBJECT		# SUBJECT(CODIFICATION)	
DAY	MONTH	YEAR	NAME	FAMILY NAME	PLI	

SIDE EFFECTS QUESTIONNAIRE-INTENSITY

Week 0 ☐ **Week 4** ☐ **Week 8** ☐ **Week 12** ☐

Indicate if you experienced side effects listed below over the **last four weeks** and if so, how hard was the intensity.

Side effects	**Intensity**			
	None[0]	Mild[1]	Moderate[2]	Severe[3]
1. Headache	☐	☐	☐	☐
2. Anxiety	☐	☐	☐	☐
3. Fatigue / exhaustion	☐	☐	☐	☐
4. Lack of energy	☐	☐	☐	☐
5. Tend to become exhausted quickly	☐	☐	☐	☐
6. Decreased appetite	☐	☐	☐	☐
7. Increased appetite	☐	☐	☐	☐
8. Hiccup	☐	☐	☐	☐
9. Nausea	☐	☐	☐	☐
10. Vomiting	☐	☐	☐	☐
11. Indigestion	☐	☐	☐	☐
12. Stomach or abdominal pain	☐	☐	☐	☐
13. Constipation	☐	☐	☐	☐
14. Diarrhea	☐	☐	☐	☐
15. Flatulence	☐	☐	☐	☐
16. Abdominal bloating	☐	☐	☐	☐
17. Palpitations	☐	☐	☐	☐
18. Balance disorders	☐	☐	☐	☐
19. Decreased ability to concentrate	☐	☐	☐	☐
20. Flushing	☐	☐	☐	☐
21. Feeling cold	☐	☐	☐	☐
22. Joint or members pain	☐	☐	☐	☐
23. Numbness, burning or itching (feet/hands)	☐	☐	☐	☐
24. Dark or depressing thoughts	☐	☐	☐	☐
25. Other	☐	☐	☐	☐

Body Weight and Nutritional Follow-Ups

UNIVERSITY OF MANITOBA

INITIALS OF SUBJECT NAME	FAMILY NAME	# SUBJECT (CODIFICATION) PLI	

Body weight follow-up

Week	Date (day/month/year)	Weight (kg)	Comments
Screening 1			
Screening 2			
Week -2			
Week 0			
Week 1			
Week 2			
Week 3			
Week 4			
Week 8			
Week 9			
Week 10			
Week 11			
Week 12			

Phone nutritional follow-up

Week -1 : **Date :** _____

_____

_____

_____

_____

Week 7 : **Date :** _____

_____

_____

_____

_____

Drop-Out Questionnaire

UNIVERSITY OF MANITOBA

DATE OF OBSERVATION			INITIALS OF SUBJECT		# SUBJECT(CODIFICATION)	
DAY	MONTH	YEAR	NAME	FAMILY NAME		

TERMINATION

Reason for termination:

- ❑ 1 Completion of treatment period, as defined by protocol.
- ❑ Early termination (choose primary reason):
  - ❑ 2 Adverse event, specify: _____
    RCFFN *: Complete Local Serious Adverse Event (SAE) Form*
  - ❑ 3 Dislike of study product.
  - ❑ 4 At the request of the investigator.
  - ❑ 5 At the request of patients family physician or study physician.
  - ❑ 6 Protocol violation.
  - ❑ 7 Illness or health problem, specify: _____
  - ❑ 8 Patient lost to follow-up.

Investigator's statement:

I certify that all information entered in this case report form by myself, my associates or designates is complete and accurate to the best of my knowledge.

_____ _____
Investigator signature Date (Day/Month/Year)

Study Report, Sample

Richardson Centre
for Functional Foods
and Nutraceuticals

October 2012

THE IMPACT OF DAIRY CONSUMPTION ON HEALTH: A CLINICAL STUDY
PARTICIPANT STUDY REPORT

Dear [redacted]

Let us begin by thanking you for your commitment and dedication to this research study. Your contribution was of a great value and provided us with important information on the role of dairy consumption on health.

For decades, dairy products have been an everyday staple in the diets of many people in Canada. The intake of 2-3 servings of dairy foods, as part of an overall healthy-style diet, is recommended by health authorities worldwide to optimize health and nutritional status. The purpose of this Dairy Study was to examine the effect of the recommended daily intake of conventional low-fat dairy products, versus calorie-matched control products, on a selection of heart health indicators. Included in this report are your results of anthropometric measurements (weight, height, body mass index, waist circumference, and hip circumference), body composition, blood tests (lipid and inflammatory markers), blood pressure, and endothelial functions. Reference values corresponding to your age group and gender are also included.

Please be advised that the values provided in this report are part of our current research study, they are not diagnostic and only represent single time-point measurements. If you have questions regarding the content of this report, or for additional information, please do not hesitate to contact us at [redacted]

On behalf of all members of our research team, we sincerely thank you again for taking part in our study.

[redacted]

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Research Assistants

1. WEIGHT AND BMI (BODY MASS INDEX)

BMI is a tool to establish a relationship between weight status and health. It is calculated by dividing your weight (kg) by your height squared (m^2).

Height (m): 1.67

	Treatment	Weight (kg)	BMI (kg/m^2)
Diet 1 Start	Dairy	76.0	27.4
Diet 1 End	Dairy	77.6	28.0
Diet 2 Start	Control	79.3	28.6
Diet 2 End	Control	78.8	28.4

Interpretation of BMI

BMI (kg/m^2)	Health risk*
18.5-24.9	The BMI values within this interval are generally associated with good health
25*-29.9	Slightly elevated risk (+)
30-34.9	Moderately high risk (++)
35-39.9	High risk (+++)
≥40	Very high risk (++++)

** Several studies show that people whose BMI is over 25 have a higher risk of chronic diseases such as hypertension, hyperlipidemia ("high cholesterol"), cardiovascular disease and diabetes.*

2. WAIST CIRCUMFERENCE

The measurement of waist circumference gives us an index of abdominal fat. The risk of cardiovascular disease is more closely associated with obesity when body fat is localized around the abdomen. Body fat in the hips is associated with a lower risk of chronic diseases than abdominal fat.

	Treatment	Waist circumference (cm)	Hip circumference (cm)
Diet 1 Start	Dairy	80.7	98.8
Diet 1 End	Dairy	80.6	96.3
Diet 2 Start	Control	82.2	97.8
Diet 2 End	Control	91.5	100.9

Interpretation of waist circumference

Values of reference (cm)		
Women	**Men**	**Interpretation**
< 80	< 94	Desirable value
80 to 88	94 to 102	Value to monitor
> 88	> 102	Value associated with increased health risk**

** *Studies have shown that waist circumferences exceeding 80 and 102 cm for women and men, respectively, are at increased risk of developing hypertension, hyperlipidemia ("high cholesterol"), cardiovascular disease and diabetes.*

3. Body Composition

There are several methods that accurately measure body composition. The reference method is the dual-energy X-ray absorptiometry or DXA. This measurement is highly accurate and gives the total amount of bone mass, fat mass and lean mass (i.e. muscle and organs). A total of 2 reviews of body composition with DXA were performed as part of this project at the end of each treatment.

	Treatment	Bone mass (kg)	Bone density (g/cm^2)	Fat mass (kg)	% of fat	Lean body mass (kg)
Diet 1 End	Dairy	2.84	1.23	32.50	42.04	41.96
Diet 2 End	Control	2.86	1.22	33.88	43.50	41.13

Interpretation of percent (%) of fat

Values of reference		
Women	**Men**	**Interpretation**
< 25	< 15	Too thin
25 to 30	15 to 20	Desirable value
> 30	> 20	Excess fat

4. Blood Lipids and Inflammation

Cholesterol is a lipid which the body needs to produce hormones, building cell walls, and perform other important physiological processes. The blood transports cholesterol in particles, called lipoproteins, which can be compared to freight trucks that circulate in the blood and deliver cholesterol to various organs. About 80% of the cholesterol found in your body is produced by the liver, whereas the rest comes from food.

The high-density lipoprotein (**HDL**), also known as "good" cholesterol, carry cholesterol to the liver. The **higher the blood levels of HDL**, the higher it **reduces the risk** of cardiovascular disease. The low density lipoprotein (**LDL**), known as "bad" cholesterol, carries cholesterol in the blood to the organs through arteries. The **higher the LDL** in the blood, the **higher the risk** of cardiovascular disease. It is also important that the level of **triglycerides** (the total body fat in blood) is kept low because a **high level increases the risk** of developing cardiovascular disease. A high triglyceride level is associated with being overweight, excessive alcohol consumption and/or concentrated sugar.

The C-reactive protein (**CRP**) is a blood marker of inflammation, as well as a risk factor for cardiovascular disease. Obesity, physical inactivity and poor eating habits are factors that can result in a mild, but chronic "inflammatory condition".

Treatment	End of Control (A)	End of Dairy (B)	Change (B-A)	% Change	Average values of Dairy group
Total cholesterol (mmol/L)	5.75	6.32	0.57	9.9	4.94
LDL cholesterol (mmol/L)	3.97	4.51	0.54	13.6	2.93
HDL cholesterol (mmol/L)	1.13	1.13	0.00	0.0	1.45
Triglycerides (mmol/L)	1.42	1.48	0.06	4.2	1.24
CRP (mg/L)	2.73	1.89	-0.84	-30.8	2.07

Interpretation of blood lipids

Desirable values (mmol/L)		
	Women	**Men**
Total cholesterol	< 6.0	< 6.0
HDL	> 1.3	> 1.04
Triglycerides	< 1.7	< 1.7

For LDL cholesterol…

The interpretation of blood levels of LDL cholesterol is a little more complex than other types of blood lipids. The target value for LDL cholesterol depends on other risk factors present, the more a person has risk factors for cardiovascular disease, the more they must maintain low values of LDL-cholesterol. Examples of risk factors include: hypertension, smoking, being older than 55 years, being postmenopausal, and having low HDL-cholesterol and elevated total cholesterol levels.

Target values of LDL cholesterol (mmol/L) based on cardiovascular disease risk	
Low risk	< 5.0
Moderate risk	< 3.5
High risk	< 2.0

A person is at **high risk** for cardiovascular disease if they have had in the past or recently have experienced cardiovascular incidents (ex. myocardial infarction, angina, heart failure, stroke), if they suffer from diabetes, or if they have 3 or more of the cardiovascular disease risk factors mentioned above. An individual is at **moderate risk** if they have 1 or 2 cardiovascular disease risk factors. Finally, a person is at **low risk** if they do not have any of the cardiovascular disease risk factors and do not suffer from cardiovascular disease.

For CRP…

The C-reactive protein assay is used for diagnose and monitor of **inflammatory processes**. This **protein synthesized by the liver**, is an **established marker of inflammation**.

Reference values for CRP (mmol/L)	
Desirable values	< 2.0
Mild and chronic inflammation	Between 2 and 10
Acute inflammation due to a flu, infection, or injury	> 10

6

5. BLOOD PRESSURE

Blood pressure is the pressure exerted by blood on the artery walls when the heart contracts (SBP = systolic blood pressure) or relaxes (DBP = diastolic blood pressure).

Hypertension is defined as a systolic pressure above 140 mmHg and a diastolic pressure above 90 mmHg. Hypertension may predispose cardiovascular disease. Blood pressure can be affected by being overweight, consuming a diet high in salt, high alcohol consumption, smoking and stress. Regular exercise and a balanced diet low in sodium (i.e. salt) can help control blood pressure.

	Treatment	SBP (mmHg)	DBP (mmHg)
Diet 1 Start	Dairy	104.7	77.0
Diet 1 End	Dairy	108.7	75.3
Diet 2 Start	Control	103.3	68.0
Diet 2 End	Control	100.0	73.7

Interpretation of blood pressure results

Desirable values (mmHg)	
SBP (mmHg)	< 140
DBP (mmHg)	< 90

6. ENDOTHELIAL FUNCTION

Endothelial function is a measure of the flexibility of blood vessels (vasodilation). This stretching of blood vessels is necessary to encourage the passage of nutrients in the blood to different organs and for regulating blood pressure. Decreased ability of blood vessels to constrict and dilate can promote the development of cardiovascular disease. Oxidizing agents (adverse health) affect the elasticity of blood vessels and it is suggested that the presence of antioxidants in the diet (e.g. in fruits, vegetables, spices, etc.) can prevent this deterioration.

Here, we present results on the health of your blood vessels. The result is a ratio, called EndoPAT score, which represents the ability of blood vessels to dilate in response to increased blood flow, following the release of a temporary blockage of blood flow. The higher the value of EndoPAT score, the higher the ability of blood vessels to dilate. For our study, we measured endothelial function at your fingertips following the obstruction of blood flow in the arm. Since it is a new measurement technique, no clinical reference value is recognized for this measure. For now, you may compare your results with those of the whole group.

	Treatment	Your results	Average values of the group
Diet 1 End	Dairy	2.55	1.95
Diet 2 End	Control	1.66	2.02

Thank you for participating in our study!

APPENDIX III

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