

# **Effect of consuming dairy fats on circulating fatty acid profile and metabolism**

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## Abstract

Increased interest has focused on associations between dietary fatty acids and cardiovascular disease (CVD). Current findings delineating effects of consuming saturated fatty acids (SFA) from dairy on CVD risk remain controversial. The objective of this thesis was to investigate the effects of consuming two types of dairy fat, namely those from cheese and butter on, human plasma and RBC fatty acid profiles, compared with monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and carbohydrate (CHO). A secondary objective was to investigate the association between consuming these dietary fatty acids and endogenous *de novo* fatty acid synthesis. A randomized, full-feeding, crossover, single-blinded clinical trial was conducted at the Institute of Nutrition and Functional Foods (INAF), Laval University and Richardson Centre for Functional Foods and Nutraceuticals (RCFFN), the University of Manitoba. A total of 92 women and men with abdominal obesity and relative low high density lipoprotein cholesterol (HDL-C) levels were randomized into a series of 5 treatments. The duration of each treatment was 4 weeks and separated by at least 4 weeks washout period. For plasma fatty acid profile, total plasma SFA after cheese treatment was found to be higher ( $P < 0.05$ ) than after MUFA, PUFA and CHO treatments, whereas total plasma SFA after butter treatment was only found to be higher ( $P < 0.05$ ) than after MUFA and PUFA treatments. Total plasma MUFA after MUFA treatment was higher ( $P < 0.05$ ) than after all other treatments, and total plasma PUFA after PUFA treatment was higher ( $P < 0.05$ ) than after all other treatments. Unlike plasma fatty acid profile, RBC total SFA after two dairy treatments were not higher than after CHO and PUFA treatments. Consistent with the plasma fatty acid profile, RBC total MUFA after

MUFA treatment were found to be higher ( $P < 0.05$ ) than after all other treatments. Similarly, RBC total PUFA after PUFA treatment were higher ( $P < 0.05$ ) than after all other treatments. We did not detect any differences in *de novo* palmitic acid synthesis across all treatments in the present study. However, we did see a positive correlation between *de novo* palmitic acid synthesis and body fat mass. In summary, present results suggest that consuming dairy fats, from cheese or butter, can significantly modulate plasma fatty acids in a manner that increases plasma total SFA, including myristic acid (C14:0), pentadecanoic acid (C15:0), palmitic acid (C16:0) and heptadecanoic acid (C17:0). However, the effect of consuming dairy fats on RBC fatty acid profile is relatively minor. Additionally, the *de novo* fatty acid synthesis data suggests that the quality of dietary fatty acids does not associate with human endogenous fatty acid synthesis; unlike body fat mass.



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## List of abbreviations

<b>Acetyl-CoA</b>	Acetyl Co-Enzyme A
<b>BMI</b>	Body Mass Index
<b>CHO</b>	Carbohydrate
<b>CLA</b>	Conjugated Linoleic Acid
<b>CRP</b>	C Reactive Protein
<b>CETP</b>	Cholesteryl Ester Transfer Protein
<b>CVD</b>	Cardiovascular Disease
<b>D</b>	Deuterium Atom
<b>D/H</b>	Deuterium to Proton
<b>DEXA</b>	Dual Energy X-ray Absorptiometry
<b>DHA</b>	Docosahexaenoic Acid
<b>EPA</b>	Eicosapentaenoic Acid
<b>FSR</b>	Fractional Synthesis Rate
<b>GC</b>	Gas Chromatography
<b>GC-IRMS</b>	Gas Chromatography Isotope Ratio Mass Spectrometry
<b>H</b>	Hydrogen Atom
<b>HDL-C</b>	High Density Lipoprotein Cholesterol
<b>IL-10</b>	Interleukin 10
<b>IL-6</b>	Interleukin 6

<b>INAF</b>	Institute for Nutrition and Functional Foods
<b>LDL-C</b>	Low Density Lipoprotein Cholesterol
<b>LCFA</b>	Long Chain Fatty Acid
<b>Malonyl-CoA</b>	Malonyl Co-Enzyme A
<b>MCFA</b>	Medium Chain Fatty Acid
<b>MCP-1</b>	Monocyte Chemotactic Protein 1
<b>MUFA</b>	Monounsaturated Fatty Acid
<b>NP-RQ</b>	Non-protein Respiratory Quotient
<b>OEA</b>	Oleylethanolamide
<b>PUFA</b>	Polyunsaturated Fatty Acid
<b>RBC</b>	Red Blood Cell
<b>RCFFN</b>	Richardson Centre for Functional Foods and Nutraceuticals
<b>SFA</b>	Saturated Fatty Acid
<b>TC</b>	Total Cholesterol
<b>TCA</b>	The Citric Acid
<b>TE</b>	Total Energy
<b>TFA</b>	Trans Fatty Acid
<b>TG</b>	Triacylglyceride
<b>TG-FA</b>	Triacylglyceride-Fatty Acid
<b>TNF-alpha</b>	Tumor Necrosis Factor-alpha



**VLDL-TG**

Very Low Density Lipoprotein Triacylglyceride

**VLDL-C**

Very Low Density Lipoprotein Cholesterol

**VSMOW**

Vienna Standard Mean Ocean Water

# Chapter I

## Background and literature review

### 1.1 Background review

Dairy products play a major role in agricultural production. According to the US-Department of Agriculture statistics on dairy products, over one billion pounds of total cheese, 167 million pounds of butter and other dairy products were produced in the United States in 2014 (USDA, 2015). Dairy products are widely consumed worldwide, especially in North American and European societies. Regarding nutritional aspects, dairy products contain various bioactive compounds including calcium, vitamin D and other nutrients (Aslibekyan et al, 2011). Consumption of dairy products has been recommended by several dietary guidelines since dairy delivers several health benefits to humans with a perfect balance of nutrients (Abdullah et al, 2015 & Prentice, 2014).

Cardiovascular health is a concern of many health care organizations and scientists in the world today. Cardiovascular disease (CVD) is one of the high death rate metabolic diseases. Increased blood lipid levels, hypertension, obesity, maleness, and diabetes have been considered as major risk factors of CVD (Sadeghi et al, 2014 & Kritchevsky, 2002). According to the results stated by Health Canada, over 1.4 million Canadians have heart disease while 90% Canadians who are over the age of 20 y have at least one risk factor of CVD (Health Canada, 2015). The pathogenesis of CVD involves several factors which go beyond only diet and aging; however, diet is one of the easiest approaches to control the prevalence of CVD (Kritchevsky, 2002). Restriction of consumption of dietary saturated fatty acids (SFA) and cholesterol has been

considered practical approaches to prevent CVD, and have been recommended by several dietary guidelines such as the US Dietary Guidelines (Otto et al, 2012). Dairy products are major dietary sources of SFA in most populations; however, they also contain essential nutrients including vitamins and minerals which may have desirable physiological effects on the human body (Lorenzen et al, 2011 & Otto et al, 2012). For instance, one study showed that calcium from dairy products favorably modulated fecal fat excretion and blood lipid levels in humans (Rice, 2014). However, high amounts of SFA in dairy fats are still a concern.

In northwestern European societies, dairy consumption contributes to over 20% of total fat intake and 50% of SFA intake (Goldbohm et al, 2011). High intakes of dietary SFA have been considered as one of the leading causes of CVD (Goldbohm et al, 2011). Therefore, recent epidemiological studies have emphasized identifying an association between dairy fats consumption and risk of CVD (Chen et al, 2016, Abdullah et al, 2015 & Pimpin et al, 2016). However, current findings remain controversial in delineating an association between dairy fats intake and risk of CVD (Rice, 2014). Circulating fatty acid profiles not only can reflect the quality and quantity of dietary fatty acids but also associated with blood lipid levels towards modulating risk of CVD. For instance, lauric acid, myristic acid, and palmitic acid are believed to contribute to elevating unfavorable blood lipid levels (Siri-Tarino et al, 2010), whereas monounsaturated fatty acids (MUFA), largely oleic acid may have favorable effects on blood lipid profiles (Gillingham et al, 2011). However, the composition of dairy fats is unique in that it contains all of the above listed fatty acids. The primary objective of this thesis was, therefore, to investigate the effects of dairy SFA consumption on circulating fatty acid profiles, compared

with consumption of other fat types. Dietary fatty acids may also differentially modulate the synthesis and oxidation of endogenous fatty acids depending on their configurations and thus, modulate whole body energy expenditure, fat homeostasis, and risk of metabolic syndrome, including CVD. Therefore, secondary objectives were to delineate the effect of consuming different fatty acids, including dairy SFA, on human endogenous fatty acid metabolism and specifically fat synthesis.

## **1.2 Literature review**

### **1.2.1 Dairy consumption and obesity**

Obesity is defined as higher body weight, which exceeds 20% of ideal body weight or body mass index (BMI, kg/m<sup>2</sup>) over 30 in subjects (Hausman et al, 2002). Several consequences stem from obesity, including high blood pressure, type II diabetes and CVD (Warensjo et al, 2010). The prevalence of obesity in the United States has increased by 30% in the past decades, and it is still increasing in all gender, age and racial populations (Hausman et al, 2002). Studies have stated that although genetic factors contribute to the development of obesity, dietary patterns such as total energy intake and dietary fatty acids significantly associate with the development of obesity (Hausman et al, 2002).

The effects of dairy intake on weight control have been previously studied. Possible mechanisms of weight reduction from consuming dairy products may be related to reduced lipogenesis and increased lipolysis (Silva et al, 2014). In addition, calcium in dairy products may also contribute to reducing body weight in terms of increasing the amount of fecal fat excretion

(Silva et al, 2014). For instance, a meta-analysis reported that high intakes of dairy with energy restriction significantly reduced body weight (1.29 kg), body fat mass (1.11 kg) and waist circumference (2.43 cm) (Silva et al, 2014). Similarly, Warrensjo et al (2010) reported that high intakes of calcium and dairy lowered total body fat in 53 preschool children (Warrensjo et al, 2010), and Pereira et al (2002) reported that dairy consumption also reduced the risk of type II diabetes and CVD in overweight subjects.

However, the effects of consuming dairy on the development of obesity are not always consistent. For instance, Louie et al (2011) reviewed 19 cohort studies which examined relationships between dairy intake and obesity published between 1980 and 2010. Two studies reported that risk of obesity depends on the type of dairy products (Drapeau et al, 2004 & Snijder et al, 2008). Seven studies reported that dairy consumption had no significant effects on preventing the development of obesity (Louie et al, 2011). Over half of all these studies showed that dairy consumption had neither favorable nor deleterious effects. Therefore, conclusions concerning how dairy consumption relates to body weight and obesity control are difficult to draw. The pathogenesis of obesity is quite complicated since several mechanisms and factors are likely involved, including genetic, metabolic, lifestyle and environmental factors (Hausman et al, 2002). Therefore, further studies are needed so that mechanisms of development of obesity can be more clearly identified.

### **1.2.2 Dairy consumption and inflammation status**

Low grade systemic body inflammation is one of the most important risk factors of metabolic syndromes, type II diabetes and CVD (Silva et al, 2014). Cytokine reactive protein

(CRP), interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha) are major proinflammatory markers which also associate with development of CVD (Silva et al,2014). Higher intakes of dietary SFA may promote human body inflammation. Dairy products contain considerable amounts of SFA; however, dietary intake of dairy fats may also have favorable impacts.

For example, Panagiotakos et al (2010) conducted a cross-sectional study aimed to determine the association between dairy intake and inflammatory markers with 1514 men and 1528 women. Results indicated that subjects had significantly lower levels of circulating inflammatory markers after consuming 14 servings of dairy products per week than did those whom only consumed 8 servings per week (Panagiotakos et al, 2010). Similarly, Stancliffe et al (2011) indicated that levels of TNF-alpha, IL-6 and monocyte chemotactic protein 1 (MCP-1) were significantly reduced after high intakes of dairy products for a period of 12 weeks in 40 overweight subjects (Stancliffe et al, 2011).

However, current findings also remain controversial in that several studies reported no significant effects of consuming dairy products on inflammation. For instance, Labonte et al (2014) conducted a multi-centre randomized crossover study. Here, 112 adults were involved in this study which including four dietary treatments: high fat, low fat, regular fat dairy and control diet (Labonte et al, 2014). Both dairy and control groups showed reduced IL-6 levels, and adiponectin, inflammatory expression genes, and transcription factors levels were not significantly different between dairy and control treatments (Labonte et al, 2014).

Current findings delineating relationships between dairy consumption and inflammation

remain controversial. Thus, any conclusion is difficult to be drawn. Further well-designed and controlled studies are needed to investigate the impacts of dietary intake of dairy on body inflammation and other CVD risk factors.

### **1.2.3 Dairy consumption, blood lipid levels and risk of CVD**

Dairy products contain different types of fatty acids consisting of approximately 8-22% medium chain fatty acids (MCFA) and over 30% long chain SFA such as palmitic acid and stearic acid; however, a considerable amount of monounsaturated fatty acids (MUFA), as well as polyunsaturated fatty acids (PUFA), are also naturally present in dairy products (Abdullah et al, 2015). In previous sections, associations between dairy consumption and obesity or inflammation status were discussed. In addition to these CVD risk factors, blood lipid levels are one of the major risk factors associated with CVD.

Current studies have focused on relationships between levels of circulating total cholesterol (TC), triacylglycerides (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) levels, and CVD risk. An elevated LDL-C level is one of the major risk factors of CVD (Lawrence, 2013) since LDL-C can be taken up by macrophages to form foam cells which significantly contribute to the development of atherosclerosis (Mensink & Plat, 2002). The particle size of LDL-C is also associated with CVD. For instance, smaller, denser LDL-C particles easily enter the arterial wall (Kritchevsky et al, 2002). In contrast, HDL-C can take cholesterol out of tissues by a specific system combined with lecithin cholesteryl acyltransferase and cholesterol ester transfer protein (CETP) (Mensink & Plat, 2002)

and therefore, HDL-C is generally considered as “good cholesterol”.

Associations between dietary fatty acids and blood lipid levels have gained attention from scientists. Research indicates that high intakes of dietary SFA significantly elevated plasma LDL-C levels (Sonestedt et al, 2011). In particular, medium to long chain SFA such as lauric acid, myristic acid, and palmitic acid induced significant LDL-C increases in humans (Siri-Tarino et al, 2010). Studies also have shown that palmitic acid from butter significantly increases serum LDL-C levels (Tholstrup et al, 2003 & Nestel et al, 2005). Ohlsson (2010) reported that dietary palmitic acid increased LDL-C levels much more than HDL-C in plasma, and both dietary myristic acid and palmitic acid also elevated plasma TC levels. However, not all SFA are adversely associating with blood lipid levels. Evidence indicates that stearic acid has no significant influence on circulating TC and LDL-C levels, whereas it may have hypolipidemic effects (Gillingham et al, 2011, Siri-Tarino et al, 2010 & Hammad et al, 2016). Also MCFA such as caprylic acid and lauric acid exhibit higher oxidation rates than long chain SFA (Delany, 2000 & Papamandjaris et al, 1988), thus reducing body fat accumulation and enhancing energy expenditure. In addition, the two unique odd-chain SFA from dairy, pentadecanoic acid and heptadecanoic acid, reportedly provide cardioprotective effects in humans (Abdullah et al, 2015).

As described previously, dairy fats also contain a considerable amount of MUFA, largely oleic acid. Dietary oleic acid significantly reduced circulating LDL-C levels and down-regulated HMG-CoA reductase and therefore, leads to a lower *de novo* cholesterol synthesis rate (Bester et al, 2010). Substituting SFA with MUFA was found to increase HDL-C, decrease LDL-C and decrease the TC: HDL-C ratio (Gillingham et al, 2011). In addition, dairy fats also contain



linoleic acid and alpha-linolenic acid. Therefore, the unique fatty acid composition of dairy fats may differentially affect circulating lipid levels and risk of CVD than do other sources such as beef tallow.

Tholstrup et al (2003) conducted a randomized cross-over study to investigate the effects of consumption of milk, cheese, and butter on plasma lipid profiles. All diets were served to healthy young men (n=14) for a period of 4 weeks for each diet. Results showed that fasting LDL-C concentrations in plasma were significantly higher after a butter diet than a cheese diet. Similarly, Nestel et al (2005) conducted a randomized cross-over study to compare the difference in LDL-C levels between cheese and butter diets. They recruited 14 men and 5 women in this study with a period of 4 weeks for each diet. Results showed that TC was significantly increased from 5.6 to 6.1 mmol/l and the LDL-C level was significantly increased from 3.4 mmol/l to 3.9 mmol/l after a butter diet. However, no significant changes in lipid profile after the cheese diet were observed. Abdullah et al (2015) also found that plasma LDL-C level after dairy treatment was significantly higher than the isocaloric control treatment.

However, studies also reported inverse associations between dairy consumption and blood lipid levels. For instance, Josse et al (2011) conducted a randomized study with 90 participants. Participants were randomly divided into three treatments: 1) high protein, high dairy, 2) adequate protein, medium dairy, 3) adequate protein, low dairy. The duration of each treatment was 16 weeks. Results indicated that plasma TC, LDL-C, HDL-C and TG levels were not significantly different between the three treatment groups (Josse et al, 2011). Similarly, Benatar et al (2014) conducted a study with 180 participants. Participants were randomized to be increasing, reducing,

or maintaining their dairy intake for a period of 4 weeks. Results showed that there were no significant changes in plasma LDL-C, HDL-C, or TG levels or other CVD risk factors between the three treatments.

Moreover, Bohl et al (2015) conducted a long-term, randomized, parallel-controlled, and double-blinded trial with 52 participants. All subjects were randomized into 4 different treatments, namely whey protein-low MCFA, whey protein-high MCFA, casein-low MCFA, and casein-high MCFA. Results indicated that there were no significant differences in postprandial TG levels between high-MCFA and low-MCFA treatments, suggesting dietary MCFA from dairy may not affect circulating TG levels. MCFA such as caprylic acid and lauric acid undergo different metabolic pathways than other SFA (Papamandjaris et al, 1988), thus resulting in relatively lower circulating TG levels due to the rapid oxidation and cleavage rate in the body.

Taken together, dairy fatty acids are unique; however, effects of consuming dairy fats on circulating lipid profiles and CVD risk are not well-documented and fully understood. Therefore, further studies are needed to more clearly delineate effects.

#### **1.2.4 Dietary fatty acids and circulating fatty acid profile**

Chylomicrons, VLDL-C, LDL-C and HDL-C are lipoproteins that contain TG and cholesterol. Circulating fatty acid profiles reflect the quality of dietary fatty acid intake, and can also be considered biomarkers for metabolic diseases such as hyperlipidemia and CVD (Rise et al, 2007). For example, high levels of circulating myristic acid, palmitic acid and palmitoleic acid and a low level of linoleic acid contribute to increasing risk of metabolic diseases such as

insulin resistance in humans (Forsythe et al, 2007). Therefore, determining circulating fatty acid profiles is critical to understanding the association between the quality of dietary fatty acids and risk of metabolic disease including CVD.

Wardlaw et al (1990) conducted a randomized crossover study to evaluate the effects of consuming butter fat, corn oil, and high oleic sunflower oil on serum lipid levels in humans. Twenty men were recruited in the study with a duration of 7 weeks for each treatment. Each treatment contained 2 weeks of a butter diet followed by 5 weeks of a designated vegetable oil diet (Wardlaw et al, 1990). Both PUFA and MUFA diets significantly decreased palmitic acid levels after the 5 weeks treatment (Wardlaw et al, 1990). PUFA diets significantly increased linoleic acid levels while MUFA diets increased oleic acid levels in serum phospholipids (Wardlaw et al, 2002). There was no significant difference in the change of other fatty acids levels in serum phospholipids between MUFA and PUFA diets (Wardlaw et al, 1990). Similarly, Mekki and colleagues (2002) also conducted a clinical trial to investigate the effects of dietary intake of butter, olive oil and sunflower oil on human postprandial lipids and TG-lipoprotein profiles. According to the results, both olive oil and sunflower oil diets showed significant lower SFA levels compared with a control diet (Mekki et al, 2002). The olive oil diet showed the highest level of oleic acid in plasma, and sunflower oil diet showed the highest plasma level of linoleic acid (Mekki et al, 2002). Regarding the SFA profile in plasma, the change of plasma palmitic acid level was similar between the butter and control diets. Palmitic acid levels after the butter diet were significantly higher than after the olive oil and sunflower oil diets since dairy contains a considerable amount of palmitic acid (Mekki et al, 2002). However, control diets

showed a similar plasma palmitic acid level compared to the butter diet (Mekki et al, 2002). A possible explanation is that palmitic acid can be *de novo* synthesized from carbohydrates through hepatic *de novo* fatty acid synthesis; therefore, a diet rich in carbohydrates can result in a higher level of plasma palmitic acid (Abdullah et al, 2015).

In the previous case, higher levels of palmitic acid in plasma were observed after a butter diet. Palmitic acid is the predominant SFA in dairy products and the effects of palmitic acid on CVD risk remain of concern (Abdullah et al, 2015). Although dairy diets such as butter and cheese showed higher plasma SFA levels than all other diets, SFA can also be synthesized through *de novo* fatty acid synthesis; therefore, circulating fatty acid profiles may not fully represent the dietary intake of SFA (Abdullah et al, 2015). Three servings of dairy intake per day are recommended by several dietary guidelines. Abdullah and colleagues (2015) conducted a crossover clinical trial to evaluate the effects of recommended dairy products intake on circulating fatty acid profiles in healthy people. Results indicate that plasma pentadecanoic acid and heptadecanoic acid levels after dairy treatment were significantly higher than an isocaloric controlled diet (Abdullah et al, 2015). Furthermore, plasma total SFA levels after dairy treatment were significantly higher than control treatment, but no significant observations were seen in levels of MUFA and PUFA (Abdullah et al, 2015). Since pentadecanoic acid and heptadecanoic acid are odd-chain fatty acids which cannot be synthesized in human body and therefore are considered as strong biomarkers for indicating dairy fat intake.

In addition to analyzing plasma fatty acid profile, red blood cell (RBC) fatty acid profile analysis can also identify and determine the bioavailability of particular fatty acids. For instance,

Ramprasath et al (2013) conducted a double-blinded, randomized, placebo-controlled, crossover study to investigate effects of krill oil, fish oil and placebo-control treatments on plasma and RBC fatty acid profiles in 24 healthy subjects over a period of 4 weeks for each treatment and followed by a period of 8 weeks washout. Ramprasath et al (2013) reported that both plasma and RBC showed increased levels of n-3 PUFA and reduced n-6/n-3 ratio. A lower ratio of n-6/n-3 was associated with lower risk of CVD, suggesting that krill oil consumption could provide significant favorable changes on circulating fatty acid profile, and therefore towards lowering CVD risk. Meanwhile, the RBC fatty acid profile demonstrated a great bioavailability of n-3 PUFA from krill oil and efficiency of incorporation into the cell membrane (Ramprasath et al, 2013). Therefore, unlike the plasma fatty acid profile, the RBC fatty acid profile reflects the bioavailability of dietary fatty acids and can predict the long term changes in circulating fatty acid profile, so as to identify long term effects on CVD risk. However, there is no well-documented evidence delineate how dairy fats affect RBC fatty acid profile.

The circulating fatty acid profile can reflect the quality and quantity of dietary fatty acids, and also help predict body fat metabolism as well as the risk of metabolic syndrome and CVD. However, the fatty acid composition of dairy fat is quite unique and complicated, consisting of different chain lengths and degree of unsaturation of medium to long chain fatty acids. Thus, a full understanding of effects of consuming dairy fats on plasma and RBC fatty acid profile is; however, lack of well-documented evidence addressing this question, and most current studies used a semi-controlled dietary intervention trial rather than a full-feeding design. Therefore, further studies based on a longer term randomized full-feeding trial are needed to better

understand the effects of consuming dairy fats on circulating fatty acid profile and associated CVD risk factors.

### **1.2.5 Dietary fatty acids and body fat metabolism**

Dietary intake of fats provides a significant amount of energy for daily activity; however, excess energy may result in obesity. Obesity is the final result of increased fatty acid synthesis rate or *de novo* lipogenesis compared to FA oxidation or lipolysis (Hausman et al, 2002). Researchers have investigated the impact of dietary fats on whole body fat metabolism including fatty acid synthesis and oxidation (Jones et al, 2008). In foods, TG are the fats, formed with three fatty acids and one glycerol. After digestion and absorption of TG in the intestine, long chain SFA are transformed by chylomicrons and then transferred to the liver via the blood and lymph (Papamandjaris et al, 1998). However, unsaturated fatty acids and MCFA bind to albumin and are transferred to the liver directly (Papamandjaris et al, 1998). Therefore, the degree of unsaturation and chain length of fatty acids could lead to different metabolic pathways. After reaching the liver, fatty acid acetyl synthetase transforms fatty acids back to TG, which is called re-esterification, with a preference toward long chain fatty acids (LCFA) (Papamandjaris et al, 1998). In the body, the majority of lipids are oxidized by  $\beta$ -oxidation which involves several enzymes (Papamandjari et al, 1998). In each cycle of fatty acid  $\beta$ -oxidation, two carbon atoms are removed from the original fatty acid, for example, palmitoyl-CoA is oxidized to myristoyl-CoA and acetyl-CoA in one  $\beta$ -oxidation cycle. Here, ATP, carbon dioxide, FADH, and NADH are produced in each cycle. Fatty acid oxidation has been studied over decades as it

pertains to body weight control and metabolic disease prevention, and chain length and degree of unsaturation of fatty acids reportedly affect oxidation rates (Hausman et al, 2002).

Stable isotope tracer techniques were introduced to determine fatty acid oxidation and whole body metabolic rate. In 1985, Jones et al investigated differences in fatty acid oxidation rate between stearic acid, oleic acid and linoleic acid. Recovered  $^{13}\text{C}$  in exhaled carbon dioxide was measured using mass spectrometry. Results indicated that oleate showed the highest %  $^{13}\text{C}$  excretion compared with linoleate and stearate. The authors stated that equal length LCFA might have different oxidation rates (Jones et al, 1985). In this study, three fatty acids had same chain length; however, the degree of unsaturation was different. Therefore, differences in the degree of unsaturation of fatty acids may lead to different fatty acid oxidation rates (Jones et al, 1988). Jones et al (2007) conducted a crossover randomized clinical trial to investigate the difference between dietary oleic, linoleic and linolenic acids on body fat oxidation rate and energy expenditure. Participants (n=15) consumed a breakfast rich in olive oil, sunflower oil or flaxseed oil. Based on results, oleic acid showed the potential to increase fatty acid oxidation rate and body energy expenditure in the human body. Similarly, McGloy et al (2004) stated that  $^{13}\text{C}$  oleate showed the highest fatty acid oxidation rate compared to elaidate, alpha-linolenate and linoleate.

Palmitic acid is a predominant long chain SFA in dairy products. Kien et al (2005) compared the effects of dietary intake of oleic acid and palmitic acid on whole body fat oxidation rate and energy expenditure. Participants were required to consume one of the two treatments, namely a high palmitic acid diet and a high oleic acid diet. Results indicated that fatty acid oxidation rate after high palmitic acid treatment was significantly decreased from  $0.038 \pm 0.006$  g/min to 0.028

$\pm 0.005$  g/min compared to baseline. However, fatty acid oxidation rates after high oleic acid treatment were slightly increased, from  $0.040 \pm 0.006$  g/min to  $0.043 \pm 0.005$  g/min. Therefore, this finding suggested that high dietary palmitic acid intake would decrease whole body fat oxidation and daily energy expenditure, whereas high dietary oleic acid intake would have a trend that increases whole body energy expenditure, and thus be beneficial to weight maintenance. Moreover, Jones et al (1985) showed that different transport and metabolism pathways might explain the different oxidation rate between SFA and unsaturated fatty acids (Jones et al, 1985). However, even though chain length and degree of unsaturation can lead to different fatty acid oxidation rates, not all SFA showed lower oxidation rate compared with unsaturated fatty acids. Delany et al (2000) reported that lauric acid showed the highest oxidation rate followed by linolenic acid, oleic acid, linoleic acid, palmitic acid and stearic acid in their study. Dairy fat contains considerable amounts of MCFA, particularly lauric acid. Papamandjaris et al (1998) stated that after administration of MCFA and LCFA emulsions to humans, MCFA showed significant higher oxidation rates than LCFA, where the latter remained at the basal level over 10 hours. Different metabolic pathways of MCFA can lead to greater oxidation, including easier absorption from the small intestine, rapid lymph transportation and direct penetration through mitochondrial membranes without the use of acylcarnitine transferase (Papamandjaris et al, 1998). Thus, dietary intake of MCFA may enhance energy expenditure, and therefore be beneficial to obesity control as well as prevention of metabolic syndrome including CVD.

Dairy contains not only long chain SFA, but also considerable amounts of MCFA as well as MUFA. Studies indicated that long-term high dairy consumption increased whole body fat



oxidation, based on a trial with non-obese female participants over a period of 1 year (Warensjo et al, 2010). Therefore, consumption of dairy fat may not deliver the same adverse impacts on cardiovascular health as do other dietary SFA, since dairy contains a considerable amount of MCFA and unsaturated fatty acids (Warensjo et al, 2010). Dairy products also contain high amounts of calcium, which may favorably impact humans by decreasing lipogenesis rates or increasing fatty acid oxidation rates (Warensjo et al, 2010). Dietary fatty acid composition could significantly influence human body fat oxidation rate and energy expenditure. Studies about effects of dietary fatty acids on body fat oxidation rate are continually being investigated. However, results of SFA from dairy on body fat oxidation are not always consistent so more work is required

In addition to fatty acid oxidation, dietary fat can also influence the rate of *de novo* lipogenesis (Hausman et al, 2002). The effects of different dietary fats on *de novo* fatty acid synthesis are different. For instance, unsaturated fatty acid showed greater inhibitory effects on fatty acid synthesis rate but also depended on their chain length and degree of unsaturation (Hausman et al, 2002). Fatty acid synthetase and acetyl CoA carboxylase are two key enzymes that regulating *de novo* fatty acid synthesis (Hausman et al, 2002). Flick et al (1977) stated that safflower oil rich in linoleic acid significantly inhibited the induction of fatty acid synthetase activity, which significantly contributes to lowering fatty acid synthesis rate and increased degradation. Similarly, Wilson et al (1990) reported that safflower oil rich in linoleic acid showed greater inhibitory effects on rats hepatic lipogenesis compared with a diet rich in beef tallow. Furthermore, fatty acids containing over 18 carbon atoms and 2 double bonds can deliver

significant inhibitory effects to fatty acid synthetase. For instance, n-3 and n-6 PUFA have been shown to have lipogenic inhibitory effects (Hausman et al, 2002). Stable isotope tracer approaches have been used to measure *de novo* fatty acid synthesis. Deuterium oxide (D<sub>2</sub>O) is a stable isotope tracer that has been used in the measurement of human body *de novo* fatty acid synthesis rate. Leitch and Jones (1993) reported that incorporation of hydrogen atoms into fatty acids can only happen during *de novo* fatty acid synthesis or during the elongation process since hydrogen atoms cannot be incorporated into preformed fatty acids. Thus, by using a stable isotope tracer approach, researchers can investigate oxidation and synthesis rates of different fatty acids in a more accurate and precise manner. These measurements are critical to better understand human fat metabolism and control body weight, body fat composition and related metabolic risk factors.

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## Research gap

Dairy fats contain different types of fatty acids, including MCFA, SFA, MUFA as well as PUFA. Different fatty acids can differentially affect plasma, and RBC fatty acid profiles, circulating lipid levels, as well as the risk of CVD. As previously described, dietary lauric acid, myristic acid and palmitic acid found in dairy can adversely affect circulating lipid profiles, and thus increase the risk of CVD. However, MCFA such as lauric acid also have higher oxidation rates than other fatty acids which in itself can be beneficial to the human body in terms of regulating energy expenditure. Unsaturated fatty acids in dairy, particularly oleic acid and linoleic acid, may also provide cardioprotective effects such as lipid-lowering effects. Meanwhile, not all SFA have adverse effect on cardiovascular health. For instance, stearic acid exhibits neutral and even hypolipidemic effects on circulating lipid profiles, suggesting that consumption of stearic acid would not increase the risk of CVD. Therefore, dietary fatty acids can be anticipated to differentially modulate circulating fatty acid and lipid profiles. In addition, *de novo* fatty acid synthesis and oxidation processes are crucial to maintaining body fat homeostasis, and therefore affecting whole body fat metabolism, energy expenditure as well as the development of such metabolic syndromes including CVD. Dietary fatty acids can provide potential effects toward modulating body fat metabolism including *de novo* lipogenesis and fat oxidation. As discussed previously, MCFA, MUFA, and PUFA have relative higher oxidation rates than do long chain SFA such as palmitic acid and stearic acid. Unsaturated fatty acids such as oleic acid and linoleic acid also exhibit inhibitory effects on *de novo* fatty acid synthesis, whereas SFA do not. However, there is lack of well-documented evidence demonstrating how dairy fat



consumption affects body fat metabolism as dairy fats contain all of the above fatty acids.

Taken together, how dairy fats modulate circulating fatty acid profiles and body fat metabolism is not well-documented nor fully understood. However, effects of consuming dairy fats on circulating fatty acid profile as well as CVD risk factors need to be carefully assessed and investigated in-depth. Meanwhile, most current studies were not based on a full-feeding design, whereas a full-feeding trial has better control on the compliance level of study participants, thus providing more reliable findings. To the best of our knowledge, there is no well-documented evidence demonstrating how dairy fats compare with other dietary fatty acids to influence human circulating fatty acid profiles and metabolism based on a full-feeding, long-term and crossover design with larger numbers of subjects. Therefore, to delineate the effects and underlying mechanisms, a randomized clinical study with complete diet control to compare the effect of consuming dairy fats with diets containing higher MUFA, PUFA or carbohydrate on CVD risk and fatty acids concentration and metabolism is needed.

### **Objectives**

1. To investigate the effect of consuming dairy fats on plasma and RBC fatty acid profile compared with diets high in MUFA, PUFA and carbohydrate.
2. To investigate the effect of consuming these dietary fatty acids on human *de novo* fatty acid synthesis using a stable isotope tracer.

## **Bridge to chapter II**

To understand the effect of consuming dairy fats on circulating fatty acid profiles and risk of cardiovascular disease (CVD), the DAIRY study was conducted by the Institute of Nutrition and Functional Foods (INAF), Laval University and the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN), The University of Manitoba. The following chapter includes a manuscript that provides detailed study protocol, analytical methods, and primary outcomes. Results of post-treatment circulating fatty acid profiles are presented, which reflect compliance level of study participants and provide predictions of risk of CVD.

## **Chapter II**

### **Manuscript**

#### **Effect of consuming dairy fats on circulating fatty acid profile: A randomized crossover controlled trial**

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## 2.1 Introduction

Cardiovascular disease (CVD) is a major concern of many health care organizations and scientists today since CVD is one of the high death rate metabolic diseases. Modulating dietary fat intake appears to be an effective approach to control the prevalence of CVD (Abdullah et al, 2015, Gillingham et al, 2011 & Hammad et al, 2016). Fatty acids are the key component in modulating circulating lipids profile as well as cardiovascular health (Abdullah et al, 2015). Accumulated epidemiological and observational studies reported that dietary saturated fatty acids (SFA) are positively associated with the risk of CVD such as low density lipoprotein cholesterol (LDL-C) increases effects (Hooper et al, 2015; Mozaffarain et al, 2010; Siri-Tarino et al, 2010 & Xu et al, 2006). Thus, dietary guidelines such as American Heart Association recommended a less than 7% of total daily energy intake for SFA, and Health Canada recommended a substitution of SFA with unsaturated fatty acids from vegetable oils such as canola or olive oil (American Heart Association, 2006 & Health Canada, 2015).

However, current findings as to identify the association between SFA and the risk of CVD remain controversial. SFA (ie lauric acid, palmitic acid and stearic acid) may also differentially affect blood lipid levels and risk of CVD (Otto et al, 2012). A meta-analysis of 21 cohort studies reported that there is no significant evidence for concluding the deleterious effects of SFA on CVD, and the typical long-chain SFA stearic acid showed no effects on LDL-C and total cholesterol (TC) levels (Siri-Tarino et al, 2010). Evidence also indicated that dietary intake of SFA could elevate high-density lipoprotein cholesterol (HDL-C) levels, thus lowering TC: HDL ratio; however, a 5% increase of plasma LDL-C levels was observed after a 5% substitution of

carbohydrates with SFA (Lamarche et al, 2014).

On the other hand, effects of consuming SFA on CVD risk may also depend on the food matrices and sources of dietary SFA. For instance, Otto et al (2012) indicated that a higher dietary intake of dairy SFA associated with a lower risk of CVD and when replacing 2% energy from meat SFA with dairy SFA lowered risk of CVD by 25%, whereas dietary intake of meat SFA associated with a significantly higher risk of CVD. Dairy products are rich sources of SFA, particularly of long chain SFA including palmitic acid and stearic acid, representing over 30% of its total fat content (Abdullah et al, 2015). However, unlike other sources of SFA such as red meat, considerable amounts of medium chain fatty acids (MCFA) including caprylic acid and lauric acid as well as oleic acid also naturally rich in dairy products (Abdullah et al, 2015). Studies reported that consumption of butter fat, particularly palmitic acid significantly increased serum LDL-C levels, whereas consumption of cheese did not (Tholstrup et al, 2003 & Nestel et al, 2005). A recent free-living, crossover study with 124 healthy subjects reported a slightly but significantly increases in plasma LDL-C levels after dairy diet compared with control diet (Abdullah et al, 2015). Moreover, a cross-sectional study by using 3 cohorts in US adults reported that when replacing 5% energy from dairy fat with polyunsaturated fatty acids (PUFA) lowered risk of CVD by 24% (Chen et al, 2016). However, a recent meta-analysis that included 15 prospective studies showed an inverse association between dairy fat intake and the risk of CVD, suggesting dairy fat intake would not increase risk of CVD (Chen et al, 2016). In addition, a meta-analysis that included 31 studies concluded that dairy fat consumption may be associated with reduced risk of CVD (Alexander et al, 2016).

Current findings as to define the association between dairy fat intake and risk of CVD remain controversial. Most studies have; however, overlooked how SFA from different food sources associated with the development of CVD. Therefore, to fill current research gap and delineate the association, the aim of present study was to investigate the effect of consuming two types of dairy fats, namely cheese and butter, on circulating fatty acid profiles which serves as a strong biomarker for predicting the risk of CVD and has strong association with blood lipid levels.

## **2.2 Experimental methods**

### **2.2.1 Study population**

A multi-centre randomized controlled trial was conducted at the Institute of Nutrition and Functional Foods (INAF) at Laval University and the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN) at the University of Manitoba. Adult women and men age from 18 to 65 years with abdominal obesity (as known as central obesity, defined by waist circumference) and relative low HDL-C (below age and sex-specific 75% percentiles) were recruited from Quebec City and Winnipeg for this study. At the screening phase, body weight, height, waist/hip circumference, blood pressure and blood lipids levels were measured for determining eligibility. Inclusion criterion are: waist circumference  $\geq 94$  cm for men and  $\geq 80$  cm for women (Diabetes Canada, 2017); plasma HDL-C concentration  $\leq 1.53$  mmol / L for women and  $\leq 1.34$  mmol / L for men; participants who had stable weight for 6 months before the start of the study; no previous histories of CVD, type II diabetes and monogenic dyslipidaemia; not under

any medications for lipid lowering and blood pressure lowering purposes; non-smoker. In addition, individuals who have particular dietary habits such as vegetarianism and allergy to dairy consumption, and if calculated 10-y Framingham risk of CVD (D'Agostino et al, 2008) >20% were excluded from the study. All participants were recruited through telephone, newspaper, campus advertisement and local media; eligible participants were further randomized based on a computer-generated sequence. A total of 92 women and men completed at least one phase of the study; 65 subjects completed all treatment phases. Participants who completed at least 1 phase were included in analyses. The Research Ethics Boards of the University of Manitoba and Laval University approved the study. The study was registered on ClinicalTrials.gov under identifier no. NCT02106208. All research participants were required to give written consent prior to initiating the study.

### **2.2.2 Study design and dietary treatments**

Single-blinded and isocaloric experimental diets were designed in this study, including two dairy fat enriched diets, namely cheese and butter, high-carbohydrate low-fat (CHO), high monounsaturated fatty acids (MUFA) and high PUFA diets. All participants were randomized to a series of these 5 diets. The duration of each treatment was 4 weeks and separated by at least 4 weeks washout period. Participants were required to fully comply with experimental diets on treatments, and encouraged to follow their own habitual diets during the washout periods. In the two dairy diets, cheese and butter were the two main sources of SFA, representing 13% of total energy (TE). Other nutrients were 14% TE MUFA, 5% TE PUFA, 53% TE carbohydrates and

15% TE proteins. In the CHO treatment, total fat was reduced from 32% TE to 25% TE (6% TE SFA, 14% TE MUFA and 5% TE PUFA), whereas carbohydrate content was increased to 60% TE. In the MUFA diet, 7% TE SFA was partially replaced by MUFA (6% TE SFA, 21% TE MUFA and 5% TE PUFA), largely from olive oil. Similarly, 7% TE SFA was replaced by PUFA in PUFA diet (6% TE SFA, 14% TE MUFA and 12% TE PUFA), largely from sunflower oil. All experimental diets were designed based on Canada's Food Guide Recommendations.

Daily energy requirement of each participant was calculated based on results from food frequency questionnaire (FFQ) and online food intake survey obtained at screening. Predesigned 7-day rotating menu including breakfast, lunch, and dinner was performed and cooked at clinical kitchen. On weekdays, all participants were required to visit the centre in the morning and consume breakfast under staff supervision. Cooler packed insulated bags containing lunch and dinner were provided to participants for taking home. Meals for weekends and holidays were delivered to participants' home. Body weight of each participant was monitored at daily basis through the entire duration of the study, since all treatment diets were prepared under an isocaloric condition, and therefore body weight of each participant was expected to be maintained. In addition to predesigned meals, less than 2 cups per day of caffeinated, milk and sugar-free beverages were allowed in the study. However, alcohol consumption was prohibited at the time of 2 days before scheduled measurement date. Compliance of each participant was measured by weekly food intake questionnaires and checklists. All participants were required to report any alcohol consumption and changes in medication through entire study.

### **2.2.3 Blood sample collections**



On days 1 and 2 of each dietary treatment, 20 ml fasting blood samples were collected. On days 29 and 30 of each dietary treatment, 42 ml fasting blood samples were collected. All blood samples were centrifuged at 3000 rpm for 20 min. Plasma and red blood cells (RBC) fractions were separated by centrifugation process from EDTA-contained vacutainer. Separated blood fractions were then stored at  $-80^{\circ}\text{C}$  immediately for further analysis.

#### **2.2.4 Plasma and RBC fatty acid profile analysis**

Plasma and RBC fatty acids were extracted using a verified direct transesterification method (Albert et al, 2015). 1.6 ml methanol was initially added into the sample (500  $\mu\text{l}$  plasma or 0.5 g RBC) followed by adding 75  $\mu\text{l}$  of 1mg/ml methyl cis-10-heptadecenoate (Sigma Aldrich, USA) as the internal standard. Then, adding 400  $\mu\text{l}$  toluene and 200  $\mu\text{l}$  acetyl chloride into the sample while mixed on a vortex. A ten-second nitrogen flash was also applied to each sample followed by incubation at  $80^{\circ}\text{C}$  for 1 hour. After cooling to room temperature, 5 ml of 6%  $\text{K}_2\text{CO}_3$  was added into the tube and followed by centrifugation at 2500 rpm for 5 minutes. The top layer was transferred into the vial and stored at  $-80^{\circ}\text{C}$  for further analysis.

Methylated fatty acid samples were analyzed by gas chromatography (GC, Varian 430) by using a  $30\text{m} \times 0.25\text{ mm}$  column (Agilent Technologies, Inc). The GC equipped with auto-sampler, auto-detector, and flame ionized detector. Initial temperature for the oven was preset at  $70^{\circ}\text{C}$  and hold the sample for 2 min. Then the temperature was increased as programmed: heat to  $180^{\circ}\text{C}$  at  $30^{\circ}\text{C}/\text{min}$  and hold for 1 min; heat to  $200^{\circ}\text{C}$  at  $10^{\circ}\text{C}/\text{min}$  and held for 2 min; heat to  $220^{\circ}\text{C}$  at  $2^{\circ}\text{C}/\text{min}$  and held for 8.6 min; heat to  $240^{\circ}\text{C}$  at  $50^{\circ}\text{C}/\text{min}$  and held for 10 min. Fatty acids in samples were identified based on the retention time of internal

standard used and a mix of standard known fatty acids. Concentrations of each fatty acid were expressed as percentage of total identified fatty acids.

### **2.2.5 Statistical method**

Statistical analyses were performed using SAS MIXED procedure (v9.4, Cary, NC). All results were expressed as least square means  $\pm$  standard error mean. Normality of data was observed and checked visually based on the plot of residuals. Treatment, sequence of treatment, age of participant, and sex were considered as fixed factors, whereas participant and test centre were considered as random factors. Repeated measurement of participant was also performed. Tukey-adjustment was used to account for multiple comparisons. Statistical difference was set at  $P < 0.05$  for all analyses.

### **2.3 Results**

After consumption of experimental diets, the endpoint fatty acid profiles for plasma and RBC (Table 2.4 & 2.6.) reflect the quality of fatty acid of experimental diets (Table 2.1). In Table 2.4, plasma total SFA after cheese treatment was higher ( $P < 0.05$ ) than after CHO, MUFA, and PUFA treatments. Plasma total SFA after butter treatment was only higher ( $P < 0.05$ ) than after MUFA and PUFA treatments. The predominant long chain SFA, plasma palmitic acid levels after both of cheese and butter treatments were higher ( $P < 0.05$ ) than after all other treatments. In addition, plasma myristic acid and pentadecanoic acid levels after both cheese and butter treatments were higher ( $P < 0.05$ ) than after all other treatments. Plasma heptadecanoic acid after butter treatment was higher ( $P < 0.05$ ) than after CHO, MUFA, and PUFA treatments, whereas the

level after cheese treatment was only higher ( $P<0.05$ ) than MUFA and PUFA treatments. However, the highest level of plasma stearic acid was observed after CHO treatment that significantly higher than after butter and MUFA treatments. When comparing endpoint to baseline fatty acid profile; however, no significant differences were observed for the changes of plasma total SFA between cheese, butter, and CHO treatments, and butter treatment even slightly decreased plasma total SFA by 0.58% respectively (Figure 2.3). Unlike plasma fatty acid profile, however, there were no differences of RBC total SFA between cheese, CHO, butter as well as PUFA treatments (Table 2.6). Yet RBC total SFA after cheese, CHO and butter treatments were all higher ( $P<0.05$ ) than after MUFA treatment. Levels of RBC palmitic acid were not different between cheese, butter and CHO treatments. However, consistent with plasma fatty acid profile, RBC myristic acid and pentadecanoic acid levels after both cheese and butter treatments were higher ( $P<0.05$ ) than after all other treatments. After CHO treatment, RBC stearic acid levels was higher ( $P<0.05$ ) than after MUFA and butter treatments, which was consistent with the plasma fatty acid profile. When comparing endpoint RBC fatty acid profile to baseline, a similar trend of total SFA changes to plasma was found, which were no differences between cheese, butter and CHO treatments, and butter diet slightly decreased RBC total SFA by 0.25%, respectively (Figure 2.6).

As expected, both plasma and RBC total MUFA and oleic acid after MUFA treatment were higher ( $P<0.05$ ) than after all other treatments (Table 2.4 & 2.6). When comparing endpoint to baseline, MUFA treatment increased plasma total MUFA by 21% and RBC total MUFA by 19.12%, (Figure 2.4 & 2.7, respectively). However, all other treatments also increased plasma

and RBC total MUFA from baseline. Specifically, the cheese treatment increased plasma total MUFA by 4.68% and RBC total MUFA by 8.84%, and butter treatment increased plasma total MUFA by 6.54% and RBC total MUFA by 9.98%, (Figure 2.4 & 2.7). In addition, as expected, both plasma and RBC total PUFA and linoleic acid levels after PUFA treatment were higher ( $P<0.05$ ) than after all other treatments (Table 2.4 & 2.6 respectively). When comparing endpoint to baseline fatty acid profile, cheese, butter, CHO and MUFA treatments decreased plasma total PUFA, whereas PUFA treatment increased total PUFA by 5.39%, respectively (Figure 2.5). However, unlike plasma fatty acid profile changes, all five treatments decreased total PUFA in RBC that even PUFA treatment slightly decreased RBC total PUFA by 0.88%, respectively (Figure 2.8).

## **2.4 Discussion**

Our findings are consistent with current scientific evidence that indicated that dairy fat consumption could significantly increase plasma total SFA (Weech et al, 2014 & Abdullah et al, 2015), particular myristic acid and palmitic acid (Weech et al, 2014). Current scientific opinions recommend that limiting SFA intake  $<10\%$  of total daily energy seems to be an effective approach to preventing the incidence of CVD (Vafeiadou et al, 2015 & Weech et al, 2014). However, it is hard to quantify how much total SFA in the diet should be replaced and by what types of dietary fats, and none of above studies investigated RBC tissue along plasma fatty acid profile. Unlike plasma, RBC fatty acid profile might be able to predict a long-term change in circulating fatty acid profiles since fatty acids from the plasma pool will also incorporate into cell membrane (Ramprasath et al, 2015). In RBC fatty acid profile, we did not observe

significant differences of RBC total SFA between two dairy treatments, CHO and PUFA treatments. Changes of total SFA from baseline in plasma and RBC were not significantly different between cheese, butter, and CHO treatment. However, we found that both plasma and RBC stearic acid after CHO were the highest versus all other treatments, and significantly higher than after MUFA and butter treatments. This finding is explained since dietary intake of carbohydrate would produce large amounts of glucose, and thus contribute to increased *de novo* fatty acid synthesis. Newly synthesized palmitic acid can be further elongated to stearic acid. Findings suggest that replacing SFA from dairy by carbohydrates will not significantly modulate RBC fatty acid profile. This diet could have a tendency to accumulate higher levels of SFA in RBC and higher levels of circulating stearic acid. These findings can question whether SFA should be replaced by carbohydrate and by how much?

Moreover, MCFA are naturally rich in dairy fat; however, we did not report MCFA in plasma and RBC as the concentrations of all circulating MCFA were too low to be detected. The reason for this scenario might be that our extraction protocol was unable to derivatize enough MCFA to meet the detection limit. However, lack of observation of this class of fatty acid was more likely due to the high oxidation rate of MCFA. Papamandjaris et al (1998) and Delany et al (2000) stated that MCFA such as caprylic and lauric acid showed significant higher oxidation rates than long chain SFA and even higher than MUFA and PUFA (Papamandjaris et al, 1988 & Delany et al, 2000). Rapid absorption of MCFA-TG from the small intestine, faster lymph transportation pathway and direct penetration into mitochondria without the use of acylcarnitine transferase can all contribute to increasing endogenous oxidation rate within all MCFA clusters (Papamandjaris

et al, 1998). Thus, we were unable to use circulating MCFA to indicate dairy fat intake at this stage.

However, we found plasma and RBC pentadecanoic acid after two dairy fat treatments which were significantly higher than after all other treatments. Accumulated clinical evidence suggested that pentadecanoic acid is a strong biomarker for indicating dairy fat intake since dairy is the major dietary source of pentadecanoic acid, and odd-chain SFA cannot be endogenously synthesized by human body (Abdullah et al, 2015, Yakoob et al, 2014, Santaren et al, 2014 & Otto et al, 2013). In addition to pentadecanoic acid, the 17 carbon atoms odd-chain SFA heptadecanoic acid is another biomarker for indicating dairy fat intake (Aslibekyan et al, 2012 & Liang et al, 2016). In our study, the level of plasma heptadecanoic acid after dairy treatments was also found to be higher than after non-dairy treatments. These findings are consistent with existing evidences that suggest these two odd-chain SFA can be considered as strong biomarkers of dairy fats intake. On the other hand, we also observed higher levels of plasma and RBC myristic acid after dairy treatments than after all other treatments. Myristic acid is also largely found in dairy fats (Otto et al, 2013), unlike the odd-chain SFA; however, myristic acid can be endogenously synthesized in humans. Therefore, it may compromise the ability to use myristic acid to reflect dairy fats intake. However, findings from our present study suggest that myristic acid is another possible biomarker for indicating dairy fats intake.

Our results are also consistent with previous findings that a high MUFA enriched diet can significantly increase plasma total MUFA, particularly oleic acid (Senanayake et al, 2014, Gillingham et al, 2010 & Hodson et al, 2001). Our results show that total MUFA not only can be

increased in plasma, but also increased in RBC after MUFA treatment. Interestingly, reductions of total SFA of plasma and RBC by MUFA treatment were higher than other treatments; particularly in RBC that MUFA treatment showed the highest reduction rate of total SFA than all other treatments. This result was not hard to explain, as accumulating scientific evidence suggests that dietary oleic acid can significantly contribute to improving whole body energy expenditure and body fat oxidation (Jones et al, 2008, Kien & Bunn, 2008 & Kien et al, 2005). Evidence also suggests that the derivative of oleic acid, oleoylethanolamide (OEA), was inversely associated with body fat mass gain (Pu et al, 2016 & Jones et al, 2014). In our findings, not only did MUFA treatment elevate plasma and RBC MUFA from baseline, but the CHO as well as the two dairy treatments. The possible reason is that carbohydrate intake can stimulate *de novo* fatty acid synthesis, with newly synthesized fatty acids then elongated and desaturated to oleic acid, thus increasing circulating oleic acid and MUFA levels. In contrast, dairy treatment also increased circulating total MUFA, since dairy fat naturally contain MUFA, particularly oleic acid that count for 20-30% of total dairy fat content (Abduallah et al, 2015). Meanwhile, PUFA treatment elevated plasma and RBC total MUFA from baseline as well, possibly due to the low dietary MUFA intake in participants' habitual diets, particularly due to the Western dietary pattern which contains a high amount of SFA and carbohydrate and less MUFA.

Unlike oleic acid and the n-9 group MUFA, linoleic acid is an essential fatty acid, which cannot be endogenously synthesized in humans. As expected, total PUFA and linoleic acid levels in plasma and RBC after PUFA treatment were significantly higher than all other treatments. The reduction rate of circulating total SFA after PUFA treatment was the second highest, since dietary

PUFA can also contribute to improving whole body energy expenditure and fat oxidation. (Delany et al, 2000). These findings also suggested that we had an excellent compliance level of study participants. Surprisingly, we observed a lower level of dihomo-  $\gamma$  -linolenic acid (C20:3, n-6) after PUFA treatment in both plasma and RBC. Theoretically, a high intake of linoleic acid ought to yield higher levels of circulating long chain PUFA via several desaturation and elongation steps (Sprecher et al, 1995). However, present finding is consistent with the result previously reported by Senanayake et al (2014), which plasma dihomo-  $\gamma$  -linolenic acid after a diet containing corn and safflower oil was found to be lower ( $P < 0.05$ ) than after a diet containing high-oleic canola oil (Senanayake et al, 2014). Therefore, the controversy of present findings suggests that a possible rate-limiting step may exist in the conversion process from linoleic acid to long chain PUFA, and the conversion rate may also be regulated by genetic factors among individuals (Schuchardt et al, 2016). However, there is no well-documented scientific evidence support this notion, thus further research is highly recommended.

## **2.5 Conclusion**

In conclusion, our current findings suggest that consuming two types of dairy fat, from namely cheese and butter can modulate circulating fatty acid profiles in a manner that increases plasma total SFA, myristic acid, palmitic acid, as well as the strong biomarker for indicating dairy intake, pentadecanoic acid and heptadecanoic acid. However, the effect is relatively minor on RBC fatty acid profile, particularly RBC palmitic acid after the cheese treatment was not significantly different than after all other treatments. In addition, Changes of specific FA, particularly those two odd-chain SFA from dairy fats and myristic acid reflect an excellent means



of establishing compliance level of study participants in human intervention studies. The strength of our present study is that we analyzed both plasma and RBC fatty acid profiles so that can have a better understanding of the effect of consuming different types of dietary fats on circulating fatty acid profiles over both the short and longer term. Additionally, this long-term, crossover, randomized and full-feeding trial can provide more accurate and reliable results compared to observational studies.

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**Table 2.1 Nutrient composition of experimental diets**

	<b>Cheese</b>	<b>Carbohydrate</b>	<b>MUFA</b>	<b>PUFA</b>	<b>Butter</b>
<b>Protein, % TE</b>	15	15	15	15	15
<b>CHO, % TE</b>	53	60	53	53	53
<b>Fat, % TE</b>	32	25	32	32	32
<b>SFA, % TE</b>	13	6	6	6	13
<b>MUFA, % TE</b>	14	14	21	14	14
<b>PUFA, % TE</b>	5	5	5	12	5

**TE: Total Energy**

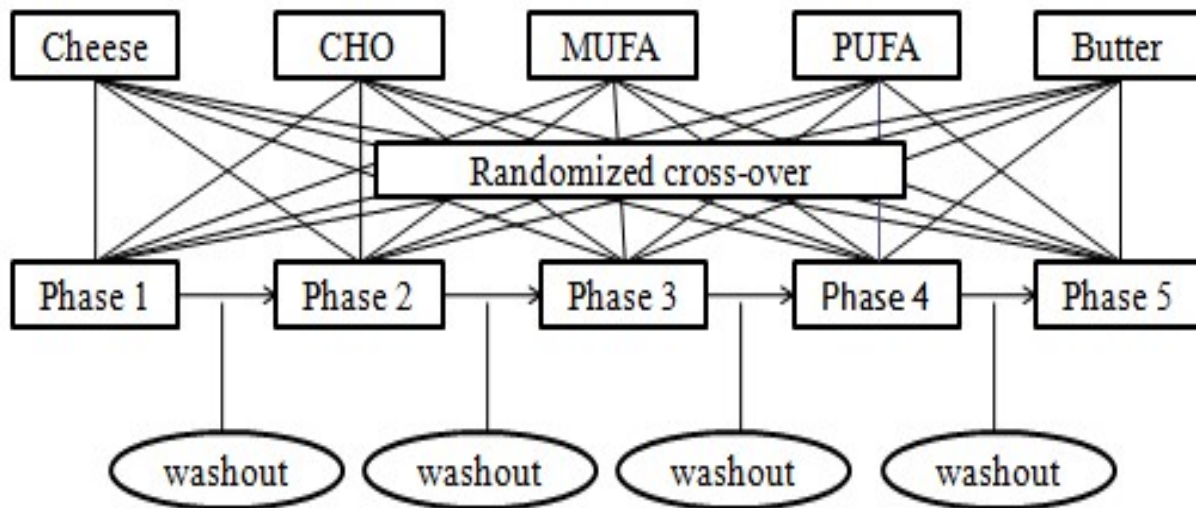
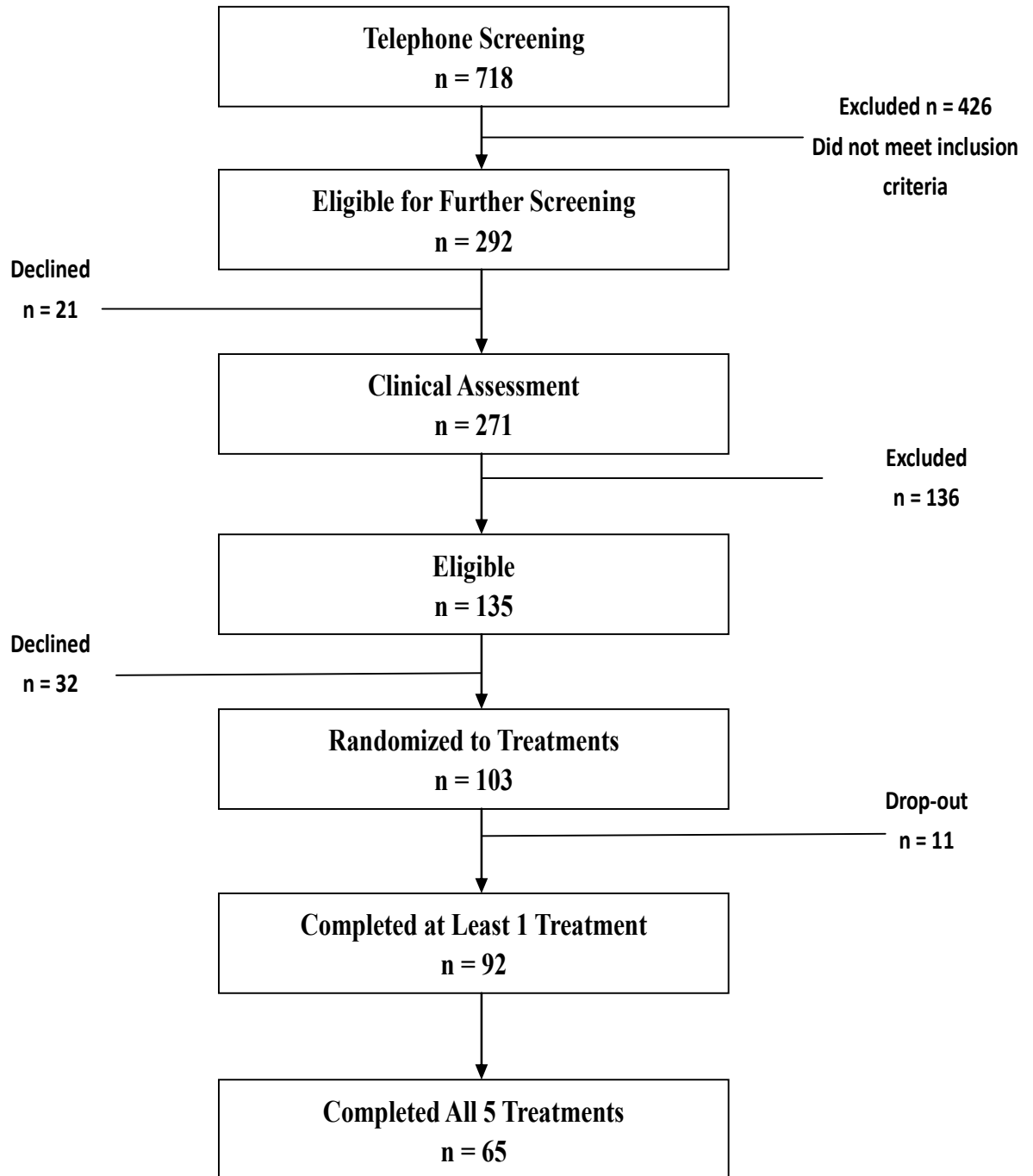


Figure 2.1 Randomized crossover design of the Study



**Figure 2.2** Flow of participants



**Table 2.2 Characteristics at screening of subjects having completed at least one diet (n=92)**

	<b>INAF (n=57)</b>	<b>RCFFN (n=35)</b>
Ethnicity, n (%)		
<i>Caucasian</i>	55 (96.5)	11 (31.4)
<i>Asian</i>	0 (0)	10 (20.0)
<i>African/African American</i>	0 (0)	7 (28.6)
<i>Hispanic</i>	1 (1.75)	6 (17.1)
<i>Other</i>	1 (1.75)	1 (2.9)
Women, n (%)	32 (56.1)	17 (48.6)
Age, y	40.6 ± 13.6	36.8 ± 13.3
Body weight, kg	86.5 ± 21.0	89.5 ± 19.8
BMI <sup>1</sup> , kg/m <sup>2</sup>	30.3 ± 6.3	31.6 ± 5.6
Waist circumference, cm	100.6 ± 14.1	103.8 ± 13.9
Plasma lipids, mmol/L		
<i>Total C</i>	5.18 ± 1.00	4.70 ± 0.81
<i>LDL-C</i>	3.22 ± 0.84	2.79 ± 0.73
<i>HDL-C</i>	1.21 ± 0.20	1.15 ± 0.22
<i>TG</i> <sup>1</sup>	1.50 ± 0.83	1.64 ± 1.17
Total cholesterol:HDL-C ratio	4.37 ± 1.01	4.28 ± 0.89
Glucose <sup>1</sup> , mmol/L	5.21 ± 0.47	5.09 ± 0.54
Blood pressure, mm Hg		
<i>Systolic</i>	113.1 ± 12.0	115.6 ± 17.0
<i>Diastolic</i>	69.4 ± 10.1	77.1 ± 10.8

Values are means ± SDs unless otherwise indicated.

<sup>1</sup> Analyses were performed on log-transformed data because original values were not normally distributed

INAF: Institute of Nutrition and Functional Foods; RCFFN: Richardson Center on Functional Foods and Nutraceuticals; BMI: body mass index; C: cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TG: triacylglyceride

**Table 2.3 Baseline plasma fatty acid profile in 92 women and men (g/100g)**

	<b>Cheese</b>	<b>Carbohydrate</b>	<b>MUFA</b>	<b>PUFA</b>	<b>Butter</b>	<b><i>P</i></b>
<b>Total SFA</b>	49.70±0.70	49.82±0.70	50.12±0.69	50.04±0.69	50.09±0.69	0.4856
<b>Total MUFA</b>	14.28±0.42	14.39±0.42	14.24±0.42	14.34±0.42	14.10±0.42	0.8552
<b>Total PUFA</b>	36.46±0.74	36.22±0.75	36.08±0.74	36.06±0.74	36.24±0.74	0.5606
<b>C14:0</b>	0.70±0.03	0.74±0.03	0.70±0.03	0.72±0.03	0.70±0.03	0.4030
<b>C15:0</b>	0.27±0.01	0.28±0.01	0.27±0.01	0.29±0.01	0.28±0.01	0.0817
<b>C16:0</b>	35.66±0.69	35.70±0.70	36.06±0.69	35.83±0.69	35.85±0.69	0.4907
<b>C16:1n7</b>	1.28±0.09	1.27±0.09	1.39±0.09	1.32±0.09	1.26±0.09	0.0560
<b>C17:0</b>	0.53±0.02	0.55±0.02	0.55±0.02	0.55±0.02	0.54±0.02	0.2617
<b>C18:0</b>	12.37±0.39	12.37±0.39	12.40±0.39	12.48±0.39	12.53±0.39	0.7682
<b>C18:1n9</b>	12.25±0.45	12.41±0.45	12.18±0.44	12.31±0.44	12.13±0.44	0.7797
<b>C18:2n6</b>	21.34±0.37	21.31±0.38	20.94±0.38	21.05±0.38	21.14±0.37	0.5198
<b>C18:3n3</b>	0.48±0.03	0.48±0.03	0.46±0.03	0.48±0.03	0.48±0.03	0.6520
<b>C20:3n6</b>	2.43±0.08	2.48±0.08	2.47±0.08	2.52±0.08	2.45±0.08	0.5055
<b>C20:4n6</b>	8.70±0.21	8.48±0.21	8.60±0.21	8.56±0.21	8.64±0.21	0.2921
<b>C20:5n3</b>	0.74±0.16	0.77±0.16	0.77±0.16	0.74±0.16	0.79±0.16	0.9051
<b>C22:5n3</b>	0.65±0.05	0.67±0.05	0.65±0.05	0.68±0.05	0.65±0.05	0.4877
<b>C22:6n3</b>	2.16±0.23 <sup>a,b</sup>	2.12±0.23 <sup>a,b</sup>	2.23±0.23 <sup>a</sup>	2.07±0.23 <sup>b</sup>	2.13±0.23 <sup>a,b</sup>	0.0841

Comparisons of five diets using the MIXED procedure; Tukey adjustment account for multiple comparisons (SAS V9.4, Cary, NC)

All values are expressed as Lsmean ± SEM

<sup>a,b,c,d</sup> means share same letter are not significantly different to each other.

**Table 2.4 Post-treatment plasma fatty acid profile in 92 women and men (g/100g)**

	<b>Cheese</b>	<b>Carbohydrate</b>	<b>MUFA</b>	<b>PUFA</b>	<b>Butter</b>	<b>P</b>
<b>Total SFA</b>	49.63±0.54 <sup>a</sup>	48.72±0.54 <sup>b</sup>	47.74±0.54 <sup>c</sup>	47.93±0.54 <sup>c</sup>	49.29±0.54 <sup>a,b</sup>	<0.0001
<b>Total MUFA</b>	14.51±0.57 <sup>b,c</sup>	15.18±0.57 <sup>b</sup>	16.74±0.56 <sup>a</sup>	14.05±0.56 <sup>c</sup>	14.57±0.56 <sup>b,c</sup>	<0.0001
<b>Total PUFA</b>	36.49±0.55 <sup>b</sup>	36.73±0.55 <sup>b</sup>	36.18±0.55 <sup>b</sup>	38.68±0.55 <sup>a</sup>	36.77±0.55 <sup>b</sup>	<0.0001
<b>C14:0</b>	0.58±0.06 <sup>a</sup>	0.46±0.06 <sup>b</sup>	0.45±0.06 <sup>b</sup>	0.46±0.06 <sup>b</sup>	0.57±0.06 <sup>a</sup>	<0.0001
<b>C15:0</b>	0.26±0.02 <sup>a</sup>	0.20±0.02 <sup>b</sup>	0.21±0.02 <sup>b</sup>	0.20±0.02 <sup>b</sup>	0.28±0.02 <sup>a</sup>	<0.0001
<b>C16:0</b>	36.06±0.61 <sup>a</sup>	35.16±0.61 <sup>b</sup>	34.73±0.61 <sup>b</sup>	34.64±0.61 <sup>b</sup>	35.93±0.61 <sup>a</sup>	<0.0001
<b>C16:1n7</b>	1.22±0.06 <sup>a</sup>	1.16±0.06 <sup>a,b</sup>	1.12±0.06 <sup>a,b</sup>	1.09±0.06 <sup>b</sup>	1.18±0.06 <sup>a,b</sup>	0.0456
<b>C17:0</b>	0.52±0.03 <sup>a,b</sup>	0.48±0.03 <sup>b,c</sup>	0.46±0.03 <sup>c</sup>	0.47±0.03 <sup>c</sup>	0.53±0.03 <sup>a</sup>	<0.0001
<b>C18:0</b>	12.87±0.19 <sup>a,b</sup>	13.05±0.19 <sup>a</sup>	12.62±0.19 <sup>b</sup>	12.80±0.19 <sup>a,b</sup>	12.58±0.19 <sup>b</sup>	0.0051
<b>C18:1n9</b>	13.36±0.44 <sup>c</sup>	14.01±0.44 <sup>b</sup>	15.64±0.44 <sup>a</sup>	12.95±0.44 <sup>c</sup>	13.45±0.44 <sup>b,c</sup>	<0.0001
<b>C18:2n6</b>	19.18±0.64 <sup>b</sup>	18.30±0.64 <sup>c,d</sup>	17.76±0.63 <sup>d</sup>	21.06±0.63 <sup>a</sup>	18.85±0.63 <sup>b,c</sup>	<0.0001
<b>C18:3n3</b>	0.39±0.04	0.37±0.04	0.38±0.04	0.37±0.04	0.40±0.04	0.6204
<b>C20:3n6</b>	2.43±0.10 <sup>a</sup>	2.52±0.10 <sup>a</sup>	2.47±0.10 <sup>a</sup>	2.13±0.10 <sup>b</sup>	2.44±0.10 <sup>a</sup>	<0.0001
<b>C20:4n6</b>	9.05±0.40 <sup>c</sup>	9.83±0.40 <sup>a</sup>	9.86±0.40 <sup>a</sup>	9.75±0.40 <sup>a</sup>	9.40±0.40 <sup>b</sup>	<0.0001
<b>C20:5n3</b>	0.80±0.11 <sup>a</sup>	0.72±0.11 <sup>a</sup>	0.82±0.11 <sup>a</sup>	0.58±0.11 <sup>b</sup>	0.83±0.11 <sup>a</sup>	<0.0001
<b>C22:5n3</b>	0.65±0.06 <sup>a,b,c</sup>	0.62±0.06 <sup>c</sup>	0.60±0.06 <sup>c</sup>	0.59±0.06 <sup>c</sup>	0.70±0.06 <sup>a</sup>	<0.0001
<b>C22:6n3</b>	3.06±0.10 <sup>b</sup>	3.32±0.11 <sup>a</sup>	3.31±0.10 <sup>a</sup>	3.10±0.11 <sup>b</sup>	3.22±0.10 <sup>a,b</sup>	<0.0001

Comparisons of five diets using the MIXED procedure; Tukey adjustment account for multiple comparisons (SAS V9.4, Cary, NC)

All values are expressed as Lsmean ± SEM

<sup>a,b,c,d</sup> means share same letter are not significantly different to each other.

**Table 2.5 Baseline RBC fatty acid profile in 92 women and men (g/100g)**

	<b>Cheese</b>	<b>Carbohydrate</b>	<b>MUFA</b>	<b>PUFA</b>	<b>Butter</b>	<b>P</b>
<b>Total SFA</b>	44.96±0.44	45.22±0.44	45.01±0.44	45.16±0.44	45.08±0.44	0.7192
<b>Total MUFA</b>	15.66±0.18	15.60±0.18	15.56±0.18	15.74±0.18	15.69±0.18	0.1733
<b>Total PUFA</b>	39.63±0.58	39.42±0.58	39.68±0.57	39.32±0.57	39.49±0.57	0.5509
<b>C14:0</b>	0.27±0.03	0.28±0.03	0.27±0.03	0.29±0.03	0.28±0.03	0.3313
<b>C15:0</b>	0.17±0.01	0.18±0.01	0.18±0.01	0.18±0.01	0.18±0.01	0.0762
<b>C16:0</b>	26.43±0.43	26.61±0.43	26.49±0.43	26.45±0.43	26.53±0.43	0.7140
<b>C16:1n7</b>	0.17±0.05	0.19±0.05	0.18±0.05	0.18±0.05	0.17±0.05	0.6087
<b>C18:0</b>	17.28±0.29	17.28±0.29	17.31±0.29	17.36±0.29	17.33±0.29	0.9718
<b>C18:1n9</b>	14.35±0.15	14.29±0.15	14.28±0.15	14.45±0.15	14.41±0.15	0.0584
<b>C18:2n6</b>	11.62±0.30	11.70±0.30	11.57±0.30	11.52±0.30	11.66±0.30	0.2964
<b>C18:3n3</b>	0.16±0.01	0.16±0.01	0.15±0.01	0.16±0.01	0.16±0.01	0.4130
<b>C20:3n6</b>	1.38±0.07 <sup>a,b</sup>	1.41±0.07 <sup>a,b</sup>	1.43±0.07 <sup>a</sup>	1.40±0.07 <sup>a,b</sup>	1.31±0.07 <sup>b</sup>	0.0556
<b>C20:4n6</b>	14.94±0.22	14.77±0.22	15.04±0.22	14.82±0.22	14.85±0.22	0.3951
<b>C20:5n3</b>	0.80±0.06	0.81±0.06	0.82±0.06	0.82±0.06	0.80±0.06	0.7279
<b>C22:5n3</b>	2.58±0.13	2.58±0.13	2.59±0.13	2.62±0.13	2.59±0.13	0.6472
<b>C22:6n3</b>	4.03±0.12	3.96±0.13	3.96±0.12	3.94±0.13	3.95±0.12	0.6040

Comparisons of five diets using the MIXED procedure; Tukey adjustment account for multiple comparisons (SAS V9.4, Cary, NC)

All values are expressed as Lsmean ± SEM

<sup>a,b,c,d</sup> means share same letter are not significantly different to each other.

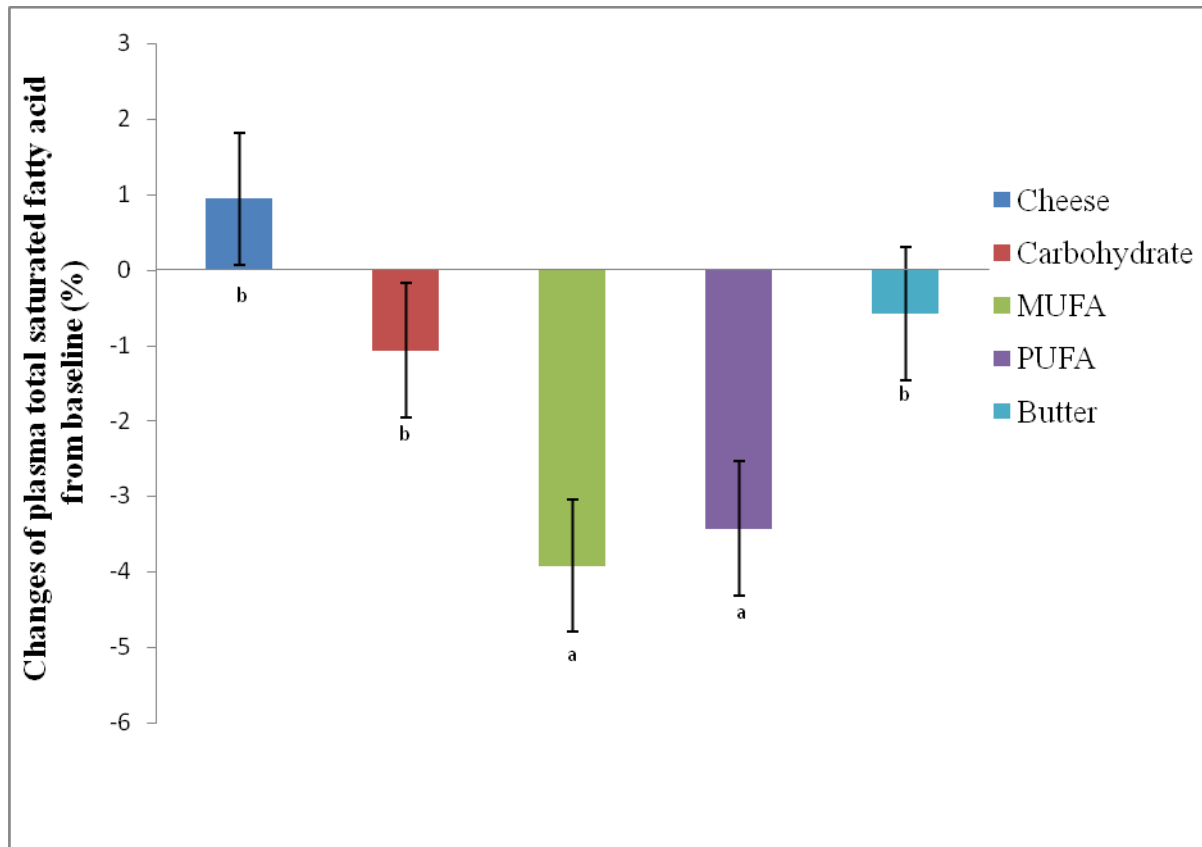
**Table 2.6 Post-Treatment RBC fatty acid profile in 92 women and men (g/100g)**

	<b>Cheese</b>	<b>Carbohydrate</b>	<b>MUFA</b>	<b>PUFA</b>	<b>Butter</b>	<b>P</b>
<b>Total SFA</b>	44.75±0.33 <sup>a</sup>	44.81±0.33 <sup>a</sup>	43.99±0.33 <sup>b</sup>	44.38±0.33 <sup>a,b</sup>	44.72±0.33 <sup>a</sup>	<0.0001
<b>Total MUFA</b>	16.70±0.34 <sup>c</sup>	17.23±0.35 <sup>b</sup>	18.20±0.34 <sup>a</sup>	16.42±0.34 <sup>d</sup>	16.91±0.34 <sup>c</sup>	<0.0001
<b>Total PUFA</b>	38.34±0.23 <sup>b</sup>	37.75±0.23 <sup>c,d</sup>	37.59±0.23 <sup>d</sup>	38.97±0.23 <sup>a</sup>	38.15±0.23 <sup>b,c</sup>	<0.0001
<b>C14:0</b>	0.28±0.02 <sup>a</sup>	0.21±0.02 <sup>b</sup>	0.19±0.02 <sup>b</sup>	0.19±0.02 <sup>b</sup>	0.29±0.02 <sup>a</sup>	<0.0001
<b>C15:0</b>	0.21±0.01 <sup>a</sup>	0.16±0.01 <sup>b</sup>	0.17±0.01 <sup>b</sup>	0.16±0.01 <sup>b</sup>	0.21±0.01 <sup>a</sup>	<0.0001
<b>C16:0</b>	25.69±0.32 <sup>a,b</sup>	25.69±0.32 <sup>a,b</sup>	25.32±0.32 <sup>b</sup>	25.41±0.32 <sup>b</sup>	25.99±0.32 <sup>a</sup>	<0.0001
<b>C16:1n7</b>	0.15±0.04 <sup>a,b</sup>	0.17±0.04 <sup>a,b</sup>	0.15±0.04 <sup>a,b</sup>	0.12±0.04 <sup>b</sup>	0.19±0.04 <sup>a</sup>	0.0151
<b>C18:0</b>	17.29±0.32 <sup>a,b</sup>	17.39±0.32 <sup>a</sup>	17.06±0.32 <sup>b,c</sup>	17.33±0.32 <sup>a,b</sup>	16.85±0.32 <sup>c</sup>	<0.0001
<b>C18:1n9</b>	14.12±0.26 <sup>c</sup>	14.59±0.26 <sup>b</sup>	15.58±0.26 <sup>a</sup>	13.89±0.26 <sup>d</sup>	14.30±0.26 <sup>c</sup>	<0.0001
<b>C18:2n6</b>	11.46±0.33 <sup>b</sup>	10.92±0.33 <sup>c</sup>	10.67±0.32 <sup>d</sup>	12.50±0.32 <sup>a</sup>	11.37±0.32 <sup>b</sup>	<0.0001
<b>C18:3n3</b>	0.10±0.01 <sup>a,b</sup>	0.10±0.01 <sup>a,b,c</sup>	0.10±0.01 <sup>b</sup>	0.09±0.10 <sup>c</sup>	0.11±0.01 <sup>a</sup>	<0.0001
<b>C20:3n6</b>	1.14±0.13 <sup>a</sup>	1.07±0.13 <sup>a,b</sup>	1.08±0.13 <sup>a,b</sup>	0.94±0.13 <sup>b</sup>	1.10±0.13 <sup>a</sup>	0.0045
<b>C20:4n6</b>	15.21±0.23	15.26±0.23	15.41±0.23	15.27±0.23	15.18±0.23	0.5942
<b>C20:5n3</b>	0.76±0.05 <sup>a,b</sup>	0.74±0.05 <sup>b</sup>	0.75±0.05 <sup>a,b</sup>	0.65±0.05 <sup>c</sup>	0.79±0.05 <sup>a</sup>	<0.0001
<b>C22:5n3</b>	2.26±0.05 <sup>a</sup>	2.23±0.05 <sup>a,b</sup>	2.20±0.05 <sup>a,b</sup>	2.18±0.05 <sup>b</sup>	2.26±0.05 <sup>a</sup>	0.0028
<b>C22:6n3</b>	4.07±0.21 <sup>a,b</sup>	4.14±0.21 <sup>a</sup>	4.09±0.21 <sup>a,b</sup>	3.97±0.21 <sup>b</sup>	4.03±0.21 <sup>a,b</sup>	0.0155

Comparisons of five diets using the MIXED procedure; Tukey adjustment account for multiple comparisons (SAS V9.4, Cary, NC)

All values are expressed as Lsmean ± SEM

<sup>a,b,c,d</sup> means share same letter are not significantly different to each other.

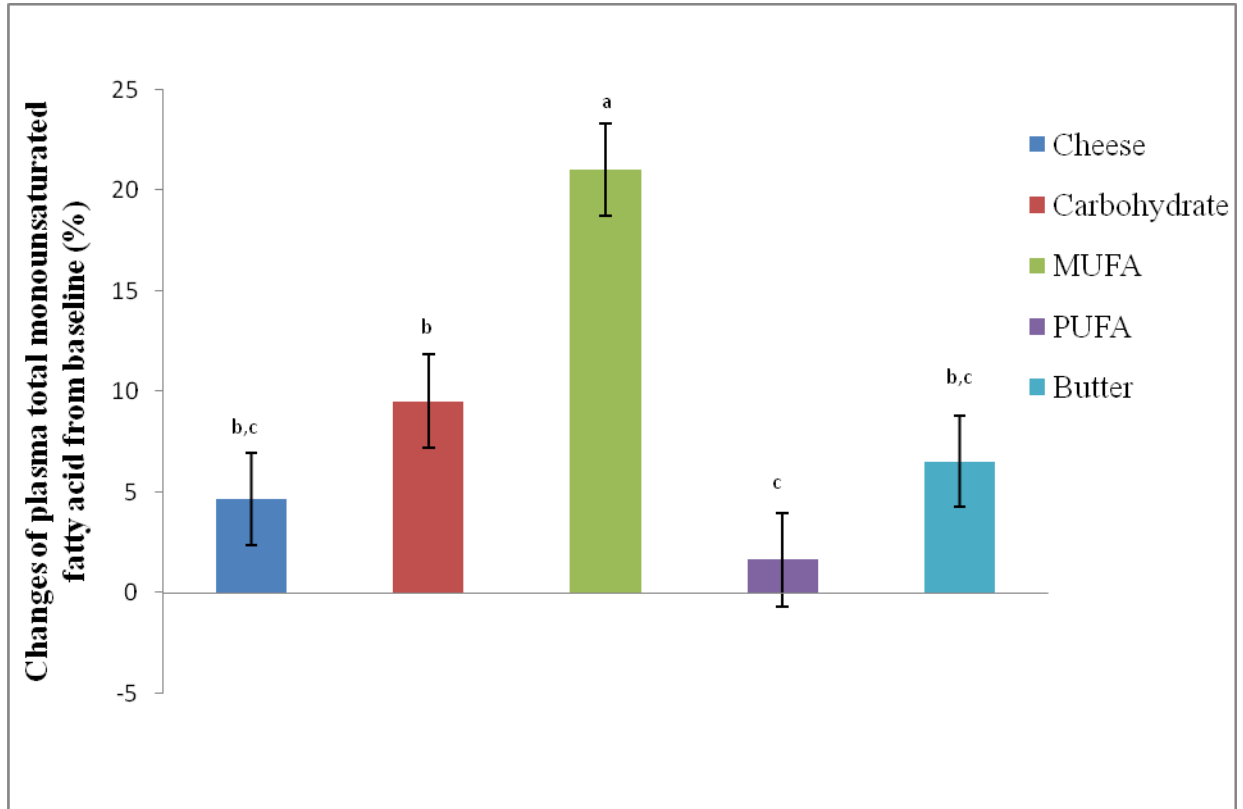


**Figure 2.3 Percent changes of plasma total saturated fatty acid from baseline in 92 women and men**

Comparisons of five diets using the MIXED procedure; Tukey adjustment account for multiple comparisons (SAS V9.4, Cary, NC)

All values are expressed as Lsmean  $\pm$  SEM

<sup>a,b</sup> means share same letter are not significantly different to each other.

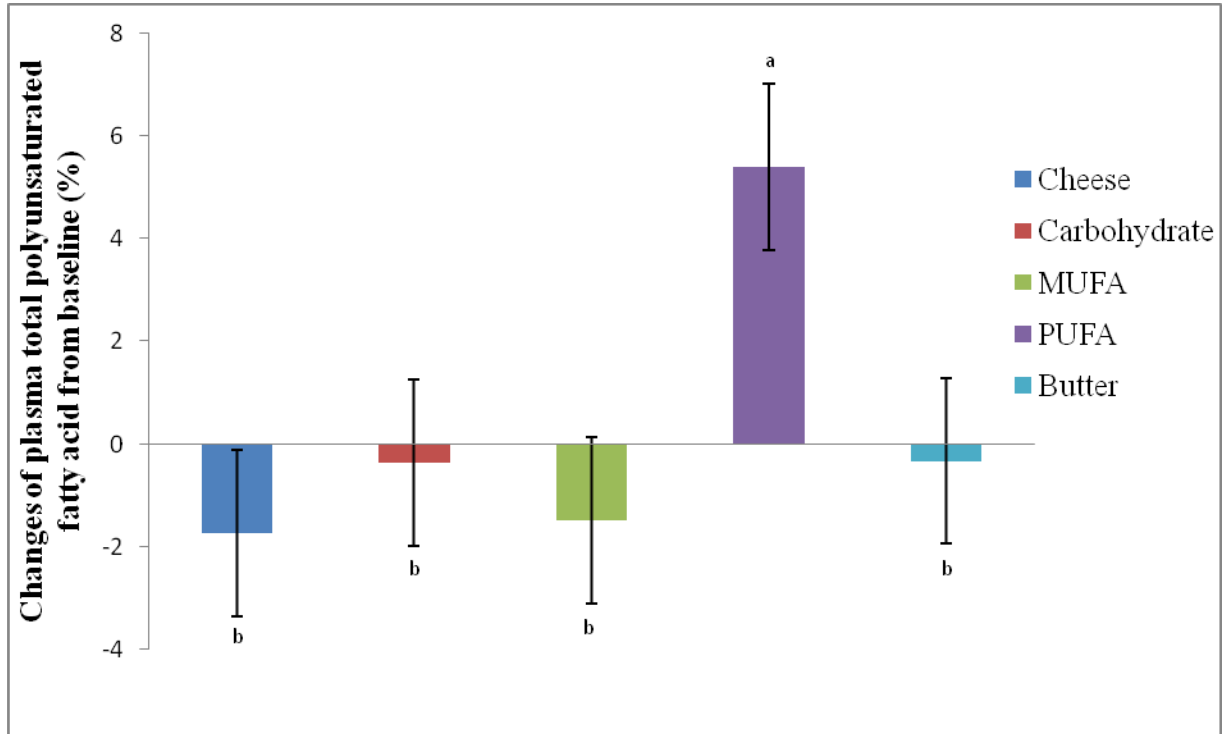


**Figure 2.4. Percent changes of plasma total monounsaturated fatty acid from baseline in 92 women and men**

Comparisons of five diets using the MIXED procedure; Tukey adjustment account for multiple comparisons (SAS V9.4, Cary, NC)

All values are expressed as Lsmean  $\pm$  SEM

<sup>a,b,c</sup> means share same letter are not significantly different to each other.



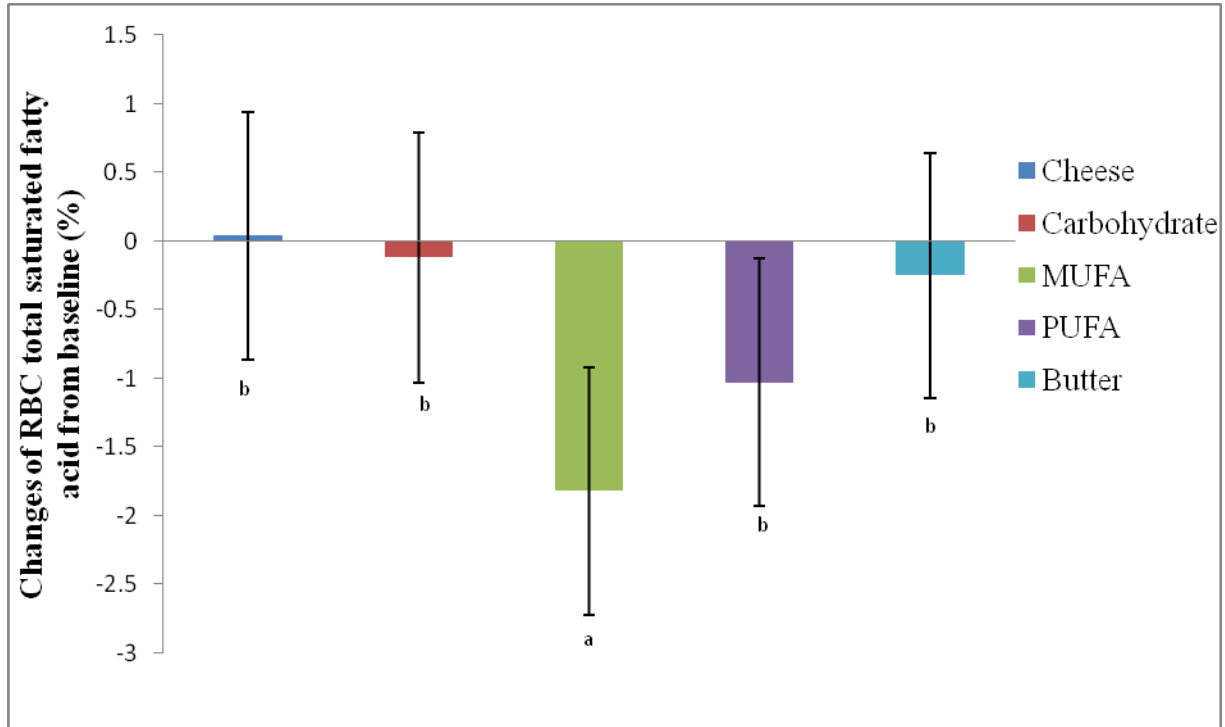
**Figure 2.5. Percent changes of plasma total polyunsaturated fatty acid from baseline in 92 women and men**

Comparisons of five diets using the MIXED procedure; Tukey adjustment account for multiple comparisons (SAS V9.4, Cary, NC)

All values are expressed as Lsmean  $\pm$  SEM

<sup>a,b</sup> means share same letter are not significantly different to each other.



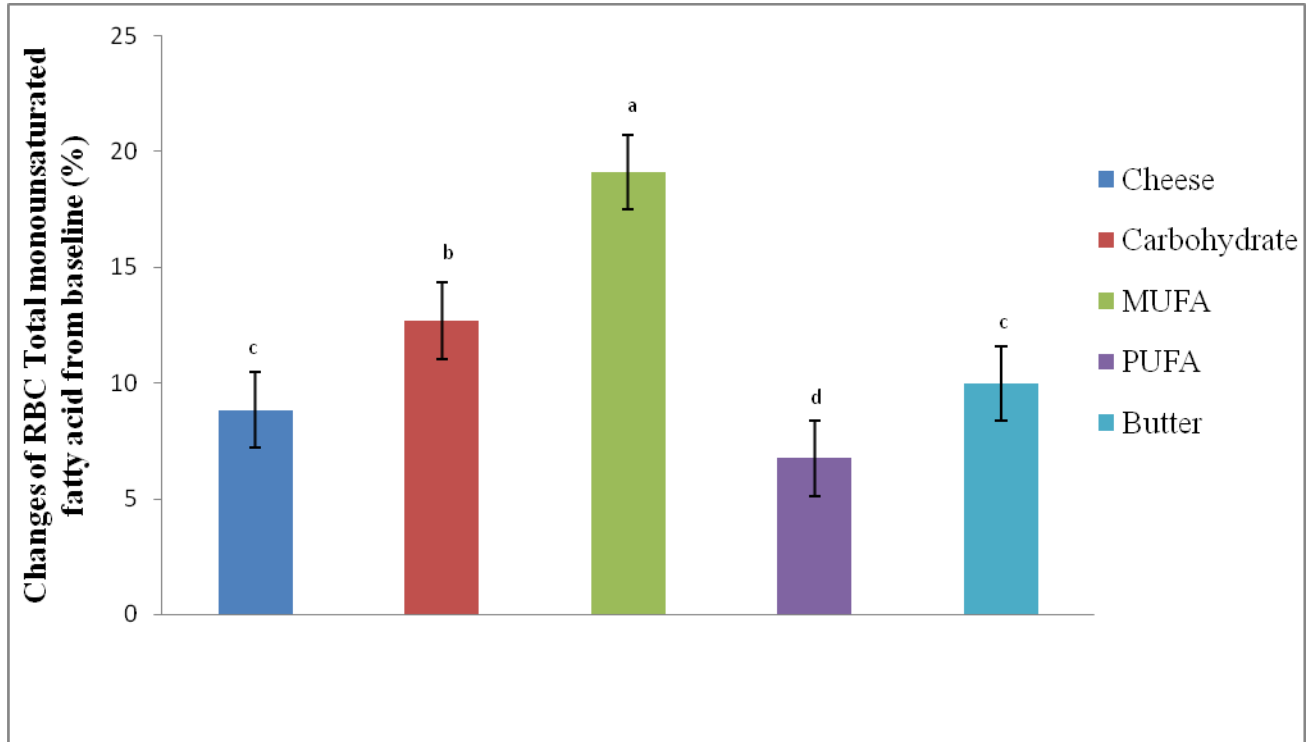


**Figure 2.6. Percent changes of RBC total saturated fatty acid from baseline in 92 women and men**

Comparisons of five diets using the MIXED procedure; Tukey adjustment account for multiple comparisons (SAS V9.4, Cary, NC)

All values are expressed as Lsmean ± SEM

<sup>a,b</sup> means share same letter are not significantly different to each other.

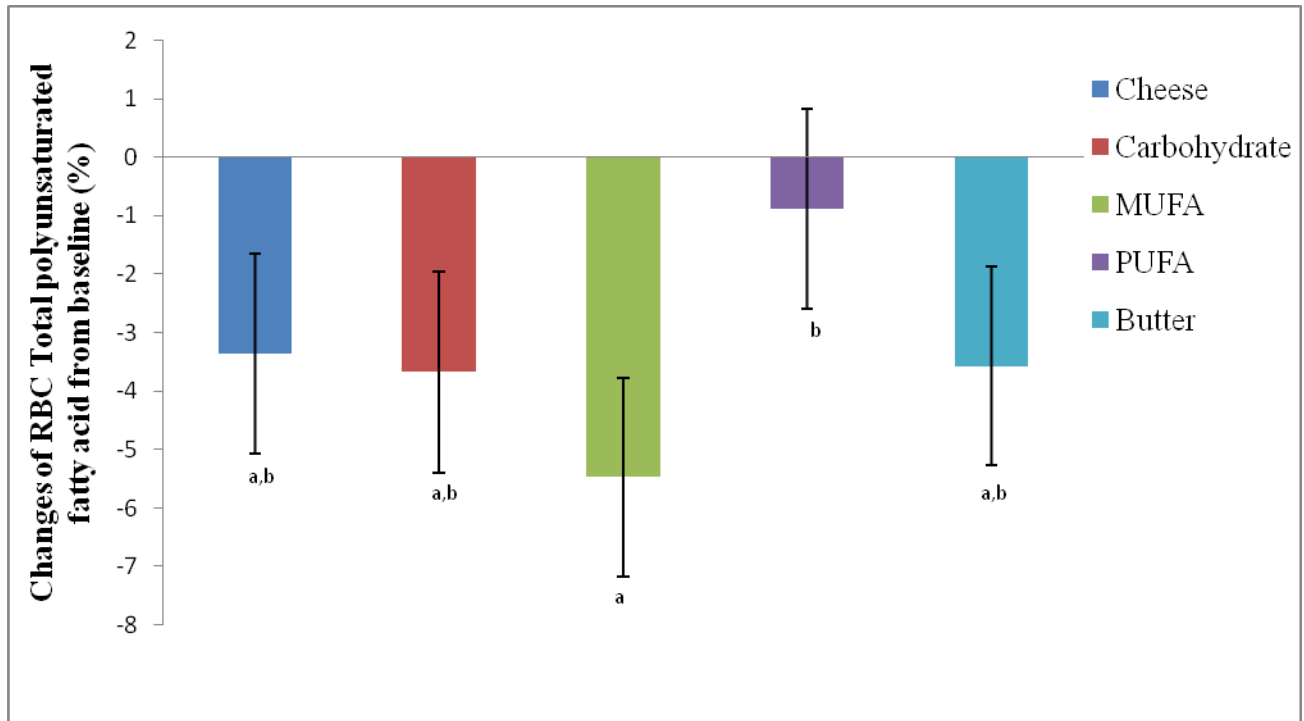


**Figure 2.7. Percent changes of RBC total monounsaturated fatty acid from baseline in 92 women and men**

Comparisons of five diets using the MIXED procedure; Tukey adjustment account for multiple comparisons (SAS V9.4, Cary, NC)

All values are expressed as Lsmean ± SEM

<sup>a,b,c,d</sup> means share same letter are not significantly different to each other.



**Figure 2.8 Percent changes of RBC total polyunsaturated fatty acid from baseline in 92 women and men**

Comparisons of five diets using the MIXED procedure; Tukey adjustment account for multiple comparisons (SAS V9.4, Cary, NC)

All values are expressed as Lsmean  $\pm$  SEM

<sup>a,b</sup> means share same letter are not significantly different to each other.

### **Bridge to chapter III**

The secondary objective of DAIRY study is to understand the effect of consuming a diet rich in dairy fats on human *de novo* fatty acid synthesis comparing with diets high in carbohydrate, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). The following chapter includes a manuscript, which provides detailed study protocol, analytical methods, and results of the secondary objective. A subgroup from the Richardson Centre for Functional Foods and Nutraceuticals consisting of 35 participants of DAIRY study are included in analyses.

## Chapter III

### Manuscript

#### **Consuming dairy fats fails to alter *de novo* fatty acid synthesis in overweight women and men**

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### 3.1 Introduction

Human *de novo* fatty acid synthesis primarily occurs in hepatic cells (Pu, et al, 2013), newly synthesized fatty acids will then be circulating in the body for several metabolic processes. *De novo* fatty acid synthesis plays a crucial role in nutritional, metabolic and physiological aspects. Enzymes such as acetyl-CoA carboxylase, acyltransferase and fatty acid synthetase are required to catalyze the process. Newly synthesized fatty acids can serve as an energy source through  $\beta$ -oxidation, precursors of longer chain fatty acids, and/or circulating in human body via hepatic very low density lipoprotein-triacylglyceride (VLDL-TG) production. However, fatty acids are the key component in modulating blood lipid levels and risk of several metabolic diseases, including obesity and cardiovascular disease (CVD). *De novo* fatty acid synthesis is believed to contribute to modulating circulating TG levels and body fat homeostasis (Lambert et al, 2013 & Leitch & Jones, 1993), thus influencing the development of several metabolic diseases. Additionally, the saturated fatty acid (SFA) palmitic acid is the final product of *de novo* fatty acid synthesis in mammal. However, elevated circulating SFA levels are considered as a risk factor of CVD (Mozaffarain et al, 2010). Therefore, it is critical to understand the metabolism of fatty acid not only at a cellular but also at a whole body level.

To measure *de novo* fatty acid synthesis, techniques such as measuring enzymatic activity, indirect calorimetry and stoichiometry have been utilized (Hellerstein, 1999). However, stable isotope tracers have gradually emerged as more useful alternatives to conventional methods in recent studies (Pu et al, 2013). Stable isotopes are a cluster of non-toxic and non-radioactive atoms which have different a number of neutrons (Jones, 1990). Clinical studies have

successfully implicated the use of deuterium oxide (D<sub>2</sub>O) in tracing human *de novo* fatty acid synthesis (Leitch et al, 1991; Letch & Jones, 1993, Jones et al, 1995; Rideout et al, 2014, Yuan et al, 2010 & Lambert et al, 2013). However, current findings appear to be controversial. The study indicated that *de novo* fatty acid synthesis is a relatively minor process, and independent from factors such as dietary pattern and nutrient composition (Jones, 1995), whereas it can only occur when total energy intake is in excess of total energy expenditure (Hellerstein, 1999). A study has shown that consumption of 500g carbohydrate per day did not elevate postprandial non-protein respiratory quotient (NP-RQ) greater than 1, which means there was virtually no *de novo* lipogenesis occurring (Acheson et al, 1982). However, others reported that *de novo* fatty acid synthesis was found to be higher after high carbohydrate diet than after high fat diet (Chong et al, 2008, Schwarz et al, 2003 & Hudgins et al, 2000), whereas high fat diet had minimal effect on *de novo* fatty acid synthesis (Hudgins, 2000).

Moreover, the majority of current studies have overlooked association between the quality of fatty acids and endogenous fatty acid synthesis. For instance, dairy fats contain high amounts of long chain SFA; however, considerable amounts of medium chain fatty acids (MCFA) as well as monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) also naturally rich in dairy (Abdullah et al, 2015 & Otto et al, 2012). Long chain PUFA such as linoleic acid may inhibit endogenous *de novo* fatty acid synthesis by down-regulating certain enzymes such as fatty acids synthetase (Flick et al, 1977 & Hausman et al, 2002), whereas SFA do not (Wilson et al, 1990). MCFA such as caprylic and lauric acid have higher endogenous oxidation rates than most of long chain fatty acids (LCFA) due to the rapid transportation from the small intestine

into hepatic cells (Papamandjaris et al, 1998 & Delany et al, 2000), thus they may have favorable effects on whole body fat metabolism. Therefore, dietary fatty acids may differentially modulate *de novo* fatty acid synthesis, depending on their chain length, degree of unsaturation and dietary sources. Unlike other food matrices such as beef tallow and vegetable oils; however, the composition of dairy fats is unique and consists all of the above fatty acids. However, there is no well-documented evidence demonstrating the effects of consuming dairy fats on endogenous fatty acid synthesis compare with other fat sources in human diet (ie: olive oil and sunflower oil).

Overall, current findings delineating an association between dietary fatty acids and *de novo* fatty acid synthesis remain unclear and controversial. It is difficult to draw a conclusion on whether the quality of fatty acids can significantly modulate *de novo* fatty acid synthesis, if so, to what extent. Therefore, to fill the current research gap and delineate the association, the aim of the present study was to investigate the effect of consuming SFA from two dairy sources namely cheese and butter on human *de novo* fatty acid synthesis compare with other nutrients (ie: MUFA, PUFA, and carbohydrate) based on a randomized trial with complete diet control.

## **3.2 Experimental methods**

### **3.2.1 Study population**

A multi-centre randomized controlled trial was conducted at the Institute of Nutrition and Functional Foods (INAF) at Laval University and the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN) at the University of Manitoba. Adult women and men age from 18 to 65 years with abdominal obesity (as known as central obesity, defined by waist circumference)



and relative low HDL-C (below age and sex-specific 75% percentiles) were recruited from Quebec City and Winnipeg for this study. At the screening phase, body weight, height, waist/hip circumference, blood pressure and blood lipids levels were measured for determining eligibility. Inclusion criterion are: waist circumference  $\geq 94$  cm for men and  $\geq 80$  cm for women (Diabetes Canada, 2017); plasma HDL-C concentration  $\leq 1.53$  mmol / L for women and  $\leq 1.34$  mmol / L for men; participants who had stable weight for 6 months before the start of the study; no previous histories of CVD, type II diabetes and monogenic dyslipidaemia; not under any medications for lipid lowering and blood pressure lowering purposes; non-smoker. In addition, individuals who have particular dietary habits such as vegetarianism and allergy to dairy consumption, and if calculated 10-y Framingham risk of CVD (D'Agostino et al, 2008)  $> 20\%$  were excluded from the study. All participants were recruited through telephone, newspaper, campus advertisement and local media; eligible participants were further randomized based on a computer-generated sequence. A total of 92 women and men completed at least one phase of the study, 35 women and men from Winnipeg area participated in the analysis of *de novo* fatty acid synthesis. Participants who completed at least 1 phase were included in analyses. The Research Ethics Boards of the University of Manitoba and Laval University approved the study. The study was registered on ClinicalTrials.gov under identifier no. NCT02106208. All research participants were required to give written consent forms prior to initiating the study.

### **3.2.2 Study design and dietary treatments**

Single-blinded and isocaloric experimental diets were designed in this study, including two

dairy fat enriched diets, namely cheese and butter, high-carbohydrate low-fat (CHO), high monounsaturated fatty acids (MUFA) and high PUFA diets. All participants were randomized to a series of these 5 diets. The duration of each treatment was 4 weeks and separated by at least 4 weeks washout period. Participants were required to fully comply with experimental diets on treatments, and encouraged to follow their own habitual diets during the washout periods. In the two dairy diets, cheese and butter were the two main sources of SFA, representing 13% of total energy (TE). Other nutrients were 14% TE MUFA, 5% TE PUFA, 53% TE carbohydrates and 15% TE proteins. In the CHO treatment, total fat was reduced from 32% TE to 25% TE (6% TE SFA, 14% TE MUFA and 5% TE PUFA), whereas carbohydrate content was increased to 60% TE. In the MUFA diet, 7% TE SFA was partially replaced by MUFA (6% TE SFA, 21% TE MUFA and 5% TE PUFA), largely from olive oil. Similarly, 7% TE SFA was replaced by PUFA in PUFA diet (6% TE SFA, 14% TE MUFA and 12% TE PUFA), largely from sunflower oil. All experimental diets were designed based on Canada's Food Guide Recommendations.

Daily energy requirement of each participant was calculated based on results from food frequency questionnaire (FFQ) and online food intake survey obtained at screening. Predesigned 7-day rotating menu including breakfast, lunch, and dinner was performed and cooked at clinical kitchen. On weekdays, all participants were required to visit the centre in the morning and consume breakfast under staff supervision. Cooler packed insulated bags containing lunch and dinner were provided to participants for taking home. Meals for weekends and holidays were delivered to participants' home. Body weight of each participant was monitored at daily basis through the entire duration of the study, since all treatment diets were prepared under an

isocaloric condition, and therefore body weight of each participant was expected to be maintained. In addition to predesigned meals, less than 2 cups per day of caffeinated, milk and sugar-free beverages were allowed in the study. However, alcohol consumption was prohibited at the time of 2 days before scheduled measurement date. Compliance of each participant was measured by weekly food intake questionnaires and checklists. All participants were required to report any alcohol consumption and changes in medication through entire study.

### **3.2.3 Stable isotope intake and analysis**

Participants received a dose of 0.7g deuterium oxide/kg body weight (99%, Cambridge Isotope Laboratories, Inc.) on day 29 of each phase. Human fatty acid synthesis was measured as the rate of deuterium incorporation from plasma water pool into newly synthesized fatty acids over 24 hours. Blood samples were collected on days 29 and 30 of each phase. Plasma and red blood cell (RBC) fractions were separated by centrifugation at 3000 rpm for 20 min from EDTA-contained vacutainer. Enrichment of deuterium on Day 29 was considered as baseline (0-hour), and day 30 was considered as endpoint (24-hour).

RBC fatty acids were extracted by verified direct transesterification method (Albert et al, 2015). 1.6 ml methanol was initially added into the sample (0.5 g RBC) followed by adding 75  $\mu$ l of 1mg/ml methyl cis-10-heptadecenoate (Sigma Aldrich, USA) as the internal standard. Then, adding 400  $\mu$ l toluene and 200  $\mu$ l acetyl chloride into the sample while mixed on a vortex. A ten-second nitrogen flash was also applied to each sample followed by incubation at 80 °C for 1 hour. After cooling to room temperature, 5 ml of 6% K<sub>2</sub>CO<sub>3</sub> was added into the tube and

followed by centrifugation at 2500 rpm for 5 minutes. The top layer was transferred into the vial and stored at -80°C for further analysis.

Extracted fatty acid methyl esters were injected into gas chromatography (GC, Agilent 6890N) isotope ratio MS (Delta V plus, Thermo Finnigan) for measuring deuterium enrichment in each sample. Fatty acids were initially separated by GC using a fused capillary column (30m × 0.25mm, Phenomenex, USA). Isolated fatty acids were reduced by a reduction reactor at the temperature of 1450°C. Reduced samples were directed into MS for measuring the ratio of deuterium to hydrogen (D/H). All samples were analyzed in duplicates to maximize accuracy. Instruments were also calibrated and maintained at a daily basis by checking parameters of background gasses, measuring linearity and stability of reference gas.

Whole body water pool size was estimated by multiplying fat-free mass by a factor of 0.73 (Yuan et al, 2010). Fat-free mass was measured on day 29 of each phase by dual-energy x-ray absorptiometry (DEXA, GE Lunar BX-1 L-8743, GE Healthcare) at RCFFN. Whole body fractional fatty acid synthesis rate (FSR) was calculated as:

$$\Delta_{\text{plasma}} = \left( \frac{R}{\text{VSMOW}} - 1 \right) \times 1000 \text{ Eq.1}$$

$$\text{FSR (\%/d)} = \frac{\Delta \text{TGFA}}{\Delta_{\text{plasma}} \times 0.477} \times 100 \text{ Eq.2}$$

The factor 0.477 was derived from the given value 0.87 g-atoms <sup>3</sup>H/g-atom C incorporated into adipose tissue fatty acids (Jungas, 1968) multiplies  $\frac{51\text{C}}{98\text{H}}$  that represent a hypothetical TG

containing three monounsaturated 17-C fatty acids as previously described by Leitch and Jones (1991). VSMOW refers to the ratio of D/H of Vienna Standard Mean Ocean Water which equals to  $1.5576 \times 10^{-4}$ .

### 3.2.4 Statistical method

Statistical analyses were performed using SAS MIXED procedure (v9.4, Cary, NC). All results were expressed as least square means  $\pm$  standard error mean. Normality of data was observed and checked visually based on the plot of residuals. Treatment, sequence of treatment, age of participant, and sex were considered as fixed factors, whereas participant was considered as random and repeated factors. Tukey-adjustment was used to account for multiple comparisons. Statistical significance was set at  $P < 0.05$  for all analyses.

### 3.3 Results

Baseline characteristics of participants are listed in Table 3.2. Forty-three participants from Winnipeg area were initially recruited and randomized. Eight participants were excluded from the study at the early stage due to elevated HDL levels, lost of interest, unable to visit RCFFN in the morning and allergy to food items from treatments. Thirty-five subjects (17 women and 18 men) who completed at least 1 phase of the study were included in analyses (Figure 3.2). All participants were either overweight or obese (BMI  $31.6 \pm 5.6 \text{ kg/m}^2$ ).

Deuterium enrichment of plasma water was estimated by calculating the molar abundance of deuterium and proton in plasma water pool using the amount of  $\text{D}_2\text{O}$  consumed and body water pool size. Calculated ratio of deuterium to proton was applied to *Eq. 1* obtaining the changes of

deuterium enrichment in plasma water over 24 hours. Figure 3.7 showed the association between observed deuterium enrichment in palmitic acid and theoretical maximum enrichment over 24 hours. Ratio of observed deuterium enrichment to theoretical maximum represents whole body fatty acid synthesis rate *Eq. 2*. Mean values of theoretical maximum enrichment ranged from  $3775.18 \pm 95.39\%$  to  $3863.94 \pm 88.68\%$ , respectively.

Calculated FSR of palmitic acid after all five treatments were not different ( $P=0.5402$ ) from each other. FSR of palmitic acid after cheese, CHO, MUFA, PUFA and butter treatments were  $2.28 \pm 0.20\%/d$ ,  $2.52 \pm 0.20\%/d$ ,  $2.58 \pm 0.20\%/d$ ,  $2.36 \pm 0.20\%/d$  and  $2.27 \pm 0.20\%/d$ , respectively (Figure 3.4). FSR of palmitic acid after MUFA treatment was found to be slightly but not significantly higher than after all other treatments. Besides, FSR values for stearic acid after all five treatments were also not different ( $P=0.7754$ ) from each other. FSR of stearic acid after cheese, CHO, MUFA, PUFA and butter treatments were  $1.52 \pm 0.10\%/d$ ,  $1.51 \pm 0.10\%/d$ ,  $1.56 \pm 0.10\%/d$ ,  $1.57 \pm 0.11\%/d$  and  $1.43 \pm 0.10\%/d$ , respectively (Figure 3.5). However, there were no trends found across FSR values of stearic acid after all five treatments.

Post-treatment correlations between FSR of palmitic acid, FSR of stearic acid and adipose tissue mass are presented in Table 3.3. Both palmitic acid stearic acid synthesis rates were negatively associated with plasma palmitic acid levels ( $r=-0.16233$ ,  $P=0.0519$ ;  $r=-0.19724$ ,  $P=0.0178$ ). However, palmitic acid synthesis rate was found to be positively associated with trunk fat mass ( $r=0.37291$ ,  $p<0.0001$ ), gynoid fat mass ( $r=0.25949$ ,  $p=0.0019$ ), android fat mass ( $r=0.38425$ ,  $p<0.0001$ ) and total body fat mass ( $r=0.33919$ ,  $p<0.0001$ ). Additionally, FSR of palmitic acid was also found to be positively correlated with FSR of stearic acid ( $r=0.35308$ ,

p<0.0001).

### 3.4 Discussion

Present results suggest that consuming dairy fats, from namely cheese and butter for a duration of 28 days does not significantly modulate *de novo* fatty acid synthesis in human body. However, we found FSR of palmitic acid after CHO treatment was slightly higher than after cheese, butter, and PUFA treatments but not significantly so. This is consistent with current scientific opinion that feeding a high carbohydrate diet can increase *de novo* lipogenesis rate due to the high production rate of pyruvate and acetyl-CoA molecules (Schutz, 2004), however, may not to a significant level. For instance, Minehira et al (2004) investigated the effects of overfeeding carbohydrate on *de novo* fatty acid synthesis in 11 lean and 8 overweight participants. Participants received a hyperenergetic diet containing 71% energy from carbohydrate. Results showed that overfeeding carbohydrate does not significantly alter *de novo* fatty acid synthesis rate (Minehira et al, 2004). In our study, weight-maintaining diet containing 60% energy from carbohydrate was given to participants. Therefore, it is reasonable that *de novo* fatty acid synthesis was not found to be significantly increased after feeding a diet rich in carbohydrate.

In addition, FSR of palmitic acid after MUFA and PUFA treatments were found to be slightly but not significantly higher than after the two dairy treatments. However, the study showed that *de novo* fatty acid synthesis rate after consuming a diet rich in SFA was found to be higher than after diet rich in unsaturated fatty acids in rats (Gnoni & Giudetti, 2016). Unsaturated fatty acids were found to have inhibitory effects on *de novo* fatty acid synthesis by inhibiting

activities such as acetyl-CoA carboxylase and fatty acid synthetase (Gnom & Giudetti, 2016).

Oleic acid and linoleic acid were also found to have higher endogenous oxidation rate than long chain SFA (Delany et al, 2000, Jones et al, 1985 & Jones et al, 2008). The coordination between malonyl-CoA and acylcarnitine transferase I has also been demonstrated that can strictly regulate the metabolism of hepatic fatty acids (Alrob & Lopaschuk, 2014, McGarry & Foster, 1979).

Therefore, these findings seem to suggest that consumption of diets rich in MUFA and PUFA can enhance whole body energy expenditure, and reduce *de novo* fatty acid synthesis. However, a potential explanation for present results may be related to the weight-maintaining situation of the study. Total fat balance ought to remain neutral on weigh-maintaining diet. Therefore, when whole body fatty acid oxidation and energy expenditure increased, *de novo* fatty acid synthesis rate may be increased in the same manner in order to maintain body fat balance. On the other hand, *de novo* fatty acid synthesis may also associate with circulating SFA levels particularly palmitic acid, which is confirmed in present results. Palmitic acid levels after dairy treatments was found to be higher ( $P<0.05$ ) than after MUFA and PUFA treatments (Chapter II). In addition, higher oxidation rate of MUFA and PUFA can also lead to a higher accumulation of acetyl-CoA molecules which may contribute to increasing fatty acid *de novo* synthesis; however, results do not show any significant associations between the quality of fatty acids and the endogenous synthesis rate at this stage.

Despite the fact that there were no significant differences of *de novo* fatty acid synthesis across all treatments observed, palmitic acid synthesis rate was found to be positively associated with the stearic acid synthesis rate and body fat mass. This is not hard to explain since stearic



acid can be elongated from palmitic acid via hepatic elongation process. Thus, synthesis rate of stearic acid was expected to be increased while the increasing of *de novo* palmitic acid synthesis. The positive correlations between palmitic acid synthesis and body fat mass suggested that *de novo* fatty acid synthesis does have an association with body fat mass; however, it is unclear whether *de novo* fatty acid synthesis affected by body fat mass, or in an opposite manner. Additionally, factors which go beyond diet may also have such effects towards regulating endogenous fat metabolism. For instance, insulin is one of the most important hormones that have been believed to regulate the process of fatty acid synthesis (Wong et al, 2010 & Kersten, 2001). A study has shown that hyperinsulinemic subjects intent to have higher lipogenesis rate than normoinsulinemic subjects (Schwarz et al, 2003). However, we are unable to identify the association at this stage due to the absence of insulin results of the present study.

In summary, our study was based on a long-term, randomized, crossover, full-feeding trial which lead to obtaining reliable results. Present findings suggest that consuming dairy fats, from namely cheese and butter for a duration of 28 days does not modulate *de novo* fatty acid synthesis in overweight women and men. Despite the fact that the quality of fatty acids does not alter *de novo* fatty acid synthesis; however, it is correlated with body fat mass. Thus, associations between factors which go beyond diet and fatty acid synthesis deserve to be investigated in future work.

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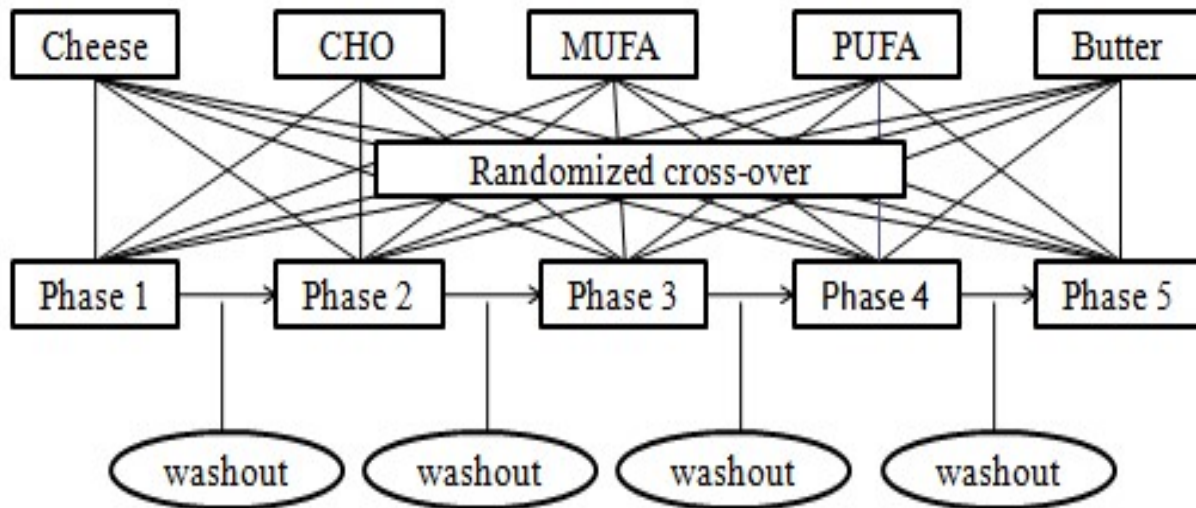
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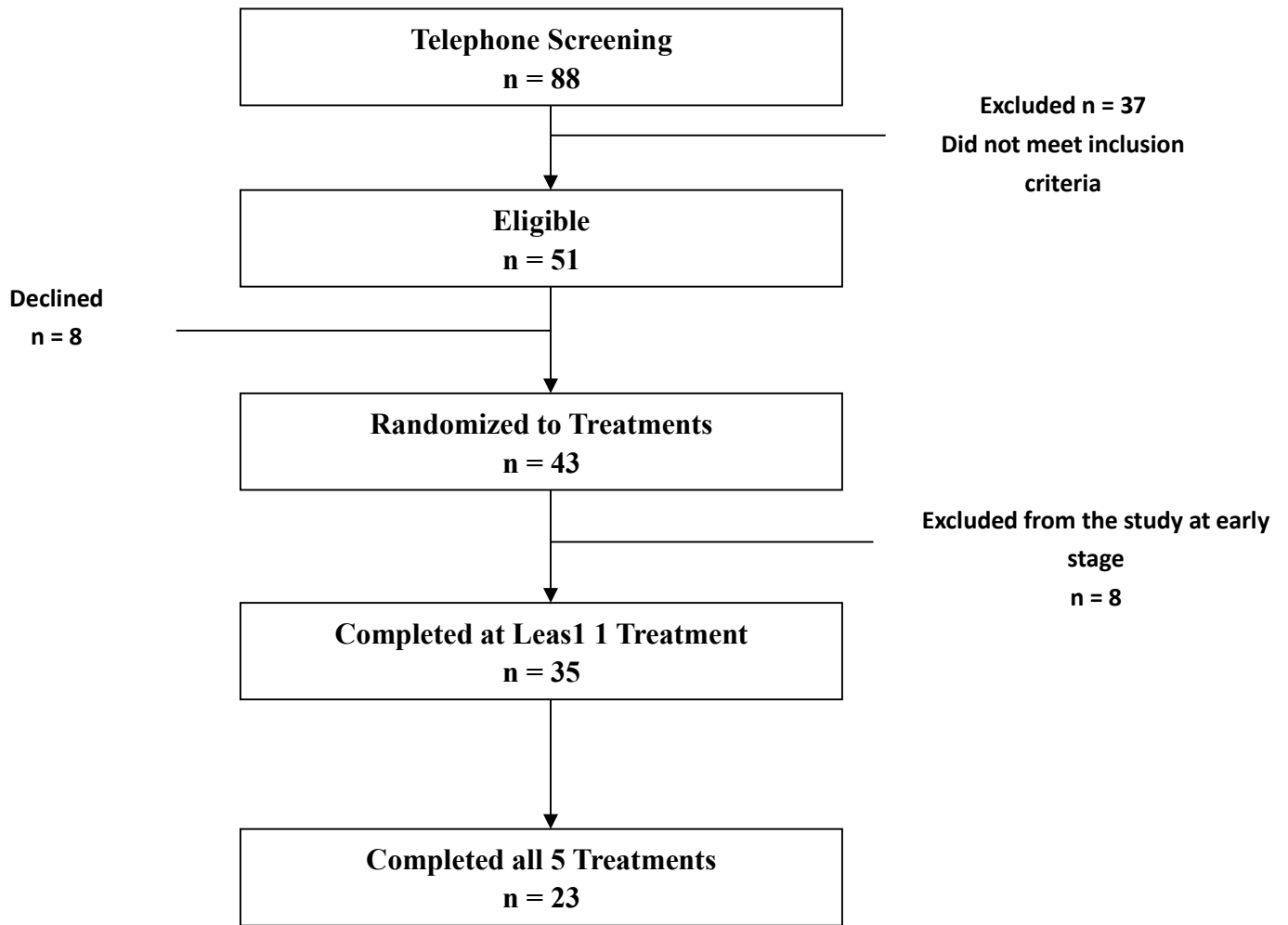
**Table 3.1 Nutrient composition of experimental diets**

	<b>Cheese</b>	<b>Carbohydrate</b>	<b>MUFA</b>	<b>PUFA</b>	<b>Butter</b>
<b>Protein, % TE</b>	15	15	15	15	15
<b>CHO, % TE</b>	53	60	53	53	53
<b>Fat, % TE</b>	32	25	32	32	32
<b>SFA, % TE</b>	13	6	6	6	13
<b>MUFA, % TE</b>	14	14	21	14	14
<b>PUFA, % TE</b>	5	5	5	12	5

**TE: Total energy**



**Figure 3.1** Randomized crossover design of the study



**Figure 3.2 Flow of participants**



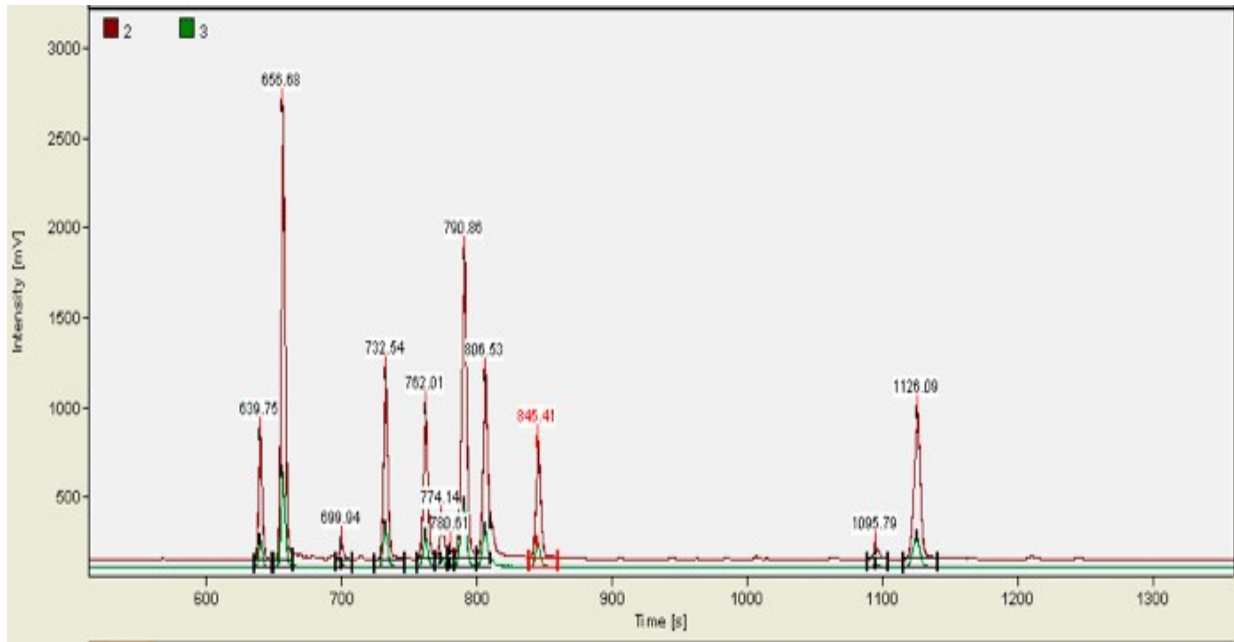
**Table 3.2 Baseline characteristics at screening of participants completed at least 1 phase (n = 35)**

	<b>RCFFN (n=35)</b>
Ethnicity, n (%)	
<i>Caucasian</i>	11 (31.4)
<i>Asian</i>	10 (20.0)
<i>African/African American</i>	7 (28.6)
<i>Hispanic</i>	6 (17.1)
<i>Other</i>	1 (2.9)
Women, n (%)	17 (48.6)
Age, y	36.8 ± 13.3
Body weight, kg	89.5 ± 19.8
BMI <sup>1</sup> , kg/m <sup>2</sup>	31.6 ± 5.6
Waist circumference, cm	103.8 ± 13.9
Plasma lipids, mmol/L	
<i>Total C</i>	4.70 ± 0.81
<i>LDL-C</i>	2.79 ± 0.73
<i>HDL-C</i>	1.15 ± 0.22
<i>TG</i> <sup>1</sup>	1.64 ± 1.17
Total cholesterol:HDL-C ratio	4.28 ± 0.89
Glucose <sup>1</sup> , mmol/L	5.09 ± 0.54
Blood pressure, mm Hg	
<i>Systolic</i>	115.6 ± 17.0
<i>Diastolic</i>	77.1 ± 10.8

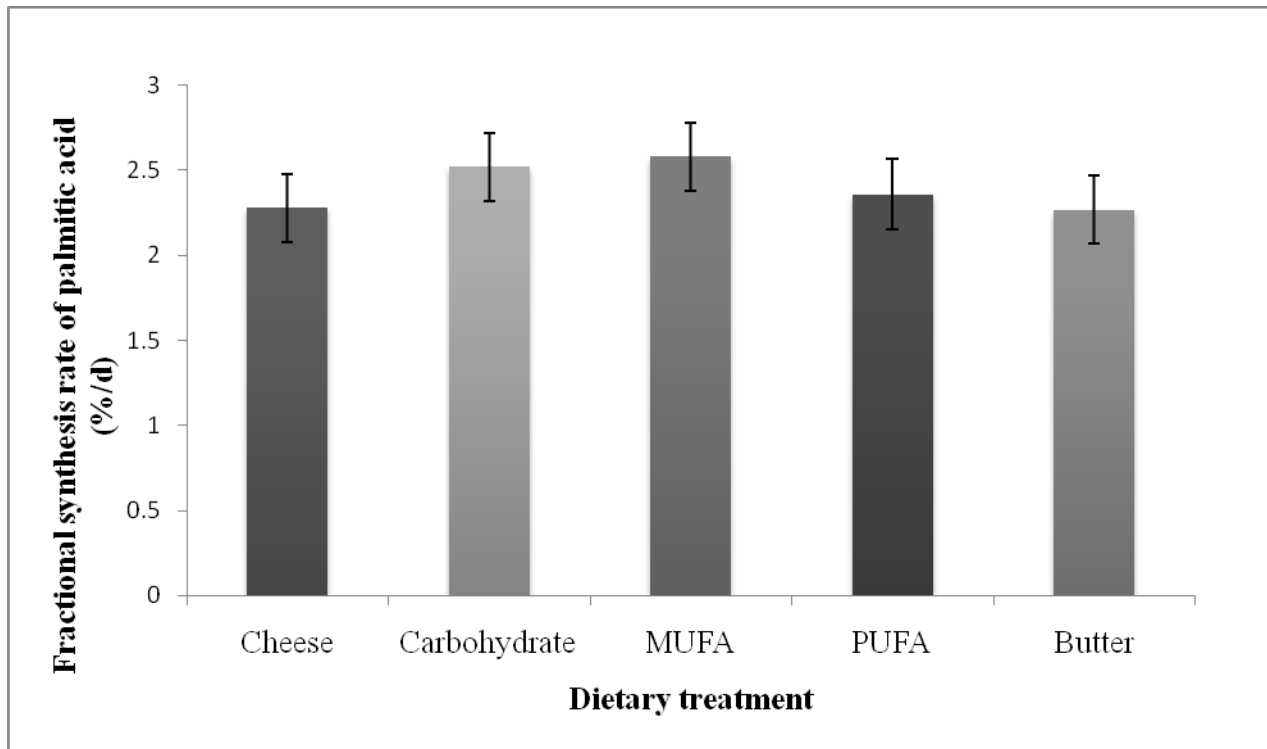
Values are means ± SDs unless otherwise indicated.

<sup>1</sup> Analyses were performed on log-transformed data because original values were not normally distributed

RCFFN: Richardson Centre for Functional Foods and Nutraceuticals



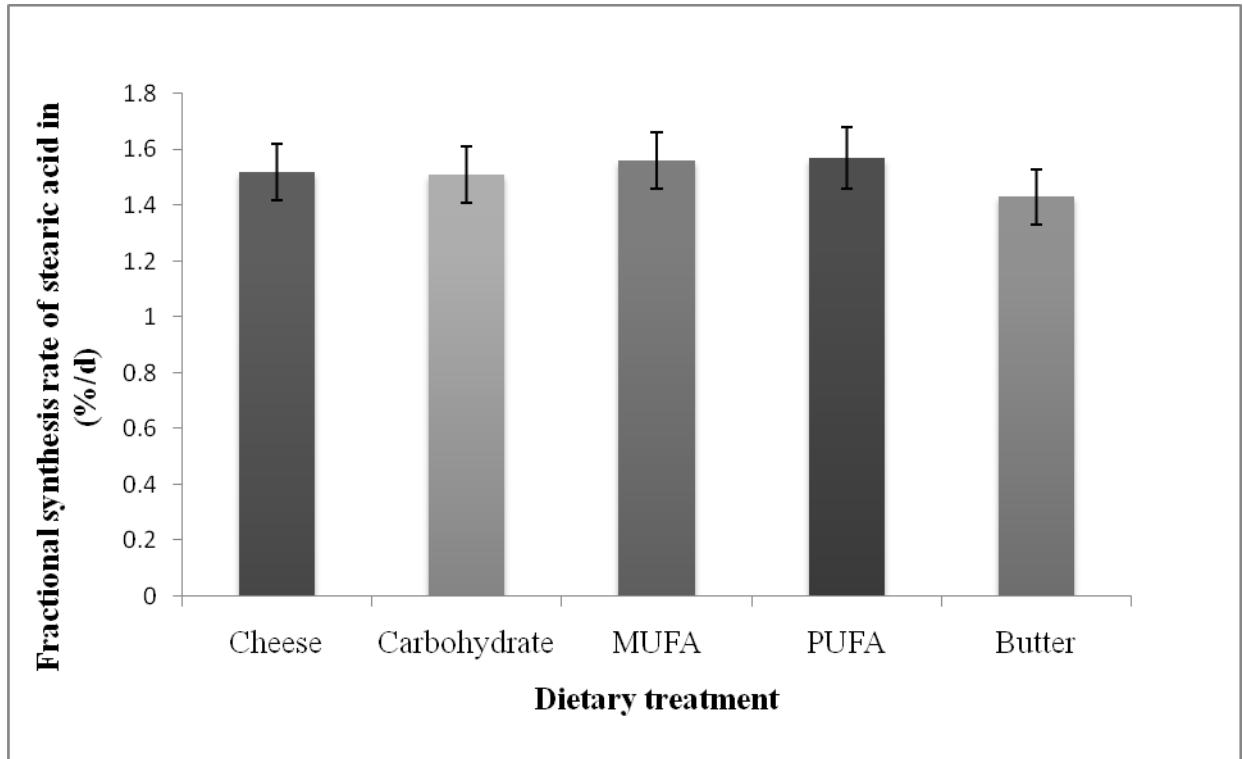
**Figure 3.3 Chromatogram of GC-IRMS for RBC fatty acid deuterium enrichment**



**Figure 3.4 Fractional synthesis rate of palmitic acid in 35 women and men**

All values are expressed as Lsmean  $\pm$  SEM

Comparisons of five diets using the MIXED procedure; Tukey adjustment account for multiple comparisons (SAS V9.4, Cary, NC)



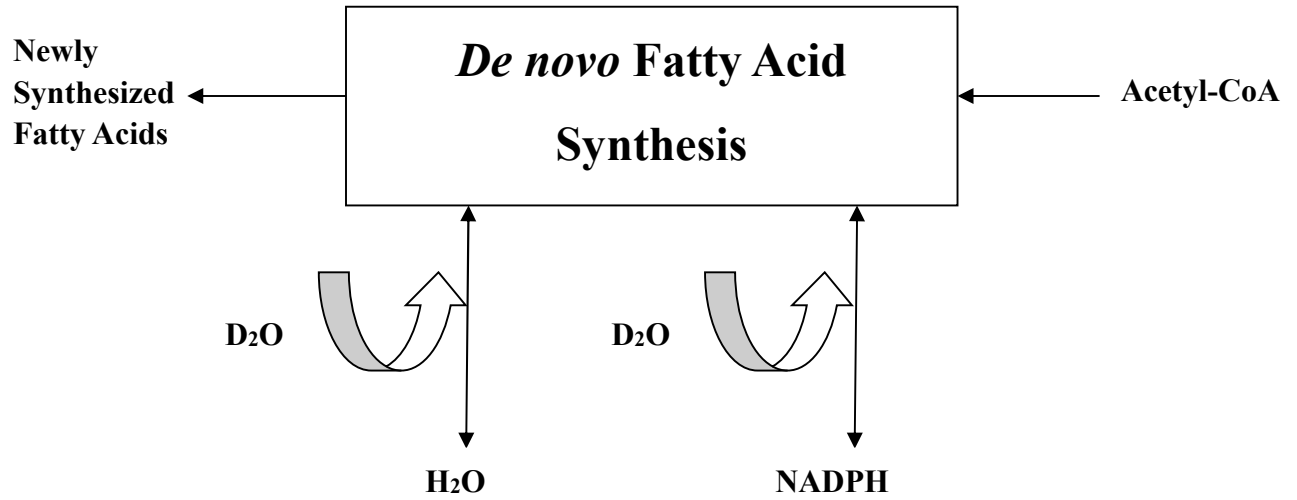
**Figure 3.5 Fractional synthesis rate of stearic acid in 35 women and men**

All values are expressed as Lsmean  $\pm$  SEM

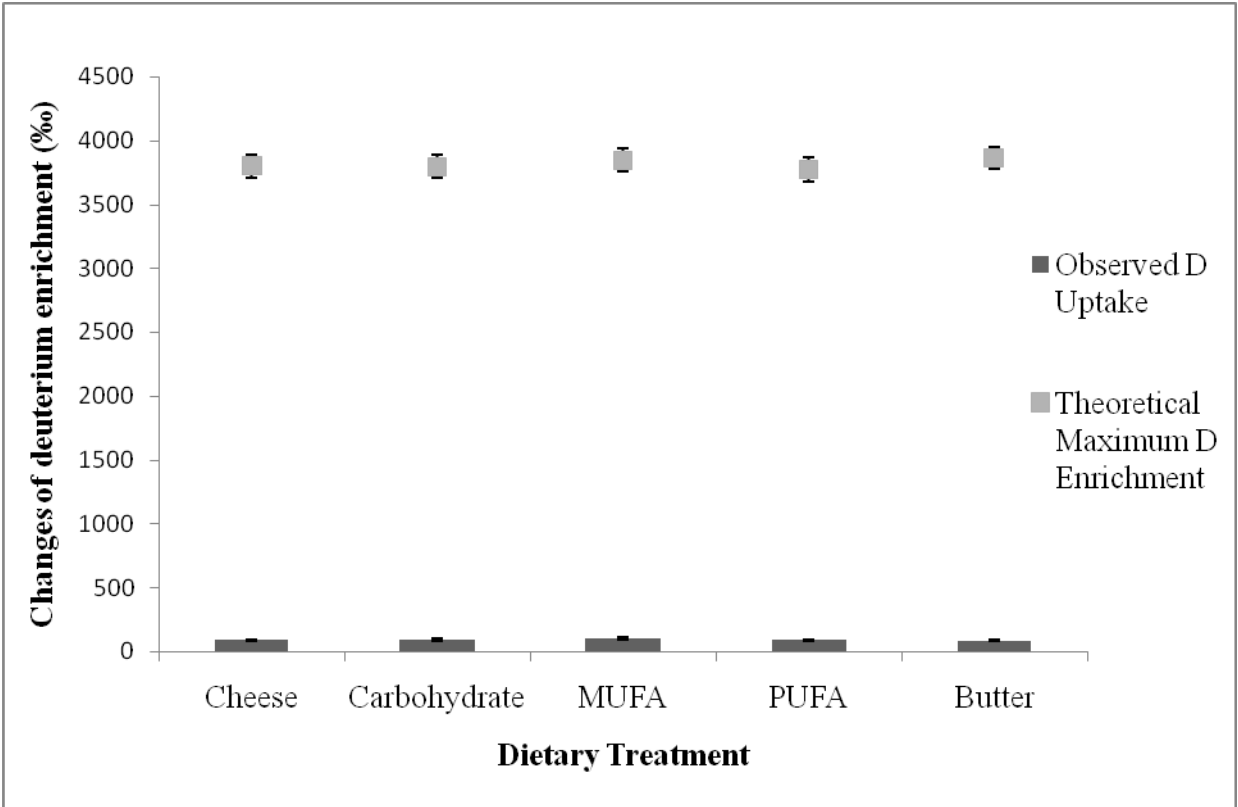
Comparisons of five diets using the MIXED procedure; Tukey adjustment account for multiple comparisons (SAS V9.4, Cary, NC)

**Table 3.3 Correlations between fatty acid synthesis, plasma palmitic acid level and body composition in 35 women and men**

	<b>Stearic acid synthesis rate</b>	<b>Plasma palmitic acid level</b>	<b>Trunk fat mass</b>	<b>Gynoid fat mass</b>	<b>Android fat mass</b>	<b>Total fat mass</b>
<b>Palmitic acid synthesis rate</b>	0.35308 P<0.0001	-0.16233 P=0.0519	0.37291 P<0.0001	0.25949 P=0.0019	0.38425 P<0.0001	0.33919 P<0.0001
<b>Stearic acid synthesis rate</b>	/	-0.19724 P=0.0178	0.05496 P=0.5175	-0.06479 P=0.4453	0.09226 P=0.2766	0.00534 P=0.9499



**Figure 3.6 Relationships between deuterium in plasma water pool and newly synthesized fatty acids**



**Figure 3.7 Observed deuterium enrichment of palmitic acid and theoretical maximum deuterium enrichment in 24 hours**

All values are expressed as Means ± SEM

## Chapter IV

### General discussion and conclusions

#### 4.1 General discussion and implications

Fatty acids are the key component in modulating blood lipid levels (Abdullah et al, 2015). Accumulated scientific evidence suggests that saturated fatty acids (SFA) particular palmitic acid, are positively associated with risk of cardiovascular disease (CVD) by adversely modulating blood lipid levels (Hooper et al, 2015; Mozaffarain et al, 2010; Siri-Tarino et al, 2010 & Xu et al, 2006). Thus, several dietary guidelines recommended a reduction in consumption of dietary SFA, however, substituting of SFA with monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA) from vegetable oils (American Heart Association, 2006 & Health Canada, 2015) is also advised.

Dairy products play a major role in the global agricultural production and human diet nowadays, which is also one of the major sources of dietary SFA. Dairy fats contain considerable amounts of SFA including palmitic acid, stearic acid as well as medium chain fatty acids (MCFA) (Abdullah et al, 2015). However, not all SFA can adversely impact blood lipid levels and risk of CVD and the effect may depend on the source of dietary fatty acids. For instance, the 18-carbon long chain SFA, stearic acid showed no effect on circulating low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) levels (Siri-Tarino et al, 2010). Studies also revealed that MCFA have high oxidation rates, which help reduce body fat accumulation while enhancing energy expenditure (Delany et al, 2000 & Papamandjaris et al, 1988). Consumption of SFA from dairy has also been demonstrated that lowers risk of CVD, but not red meat (Otto et al, 2012).



Studies also concluded an inverse relationship between dairy fats intake and risk of CVD (Chen et al, 2016 & Alexander et al, 2016); however, findings remain controversial (Tholstrup et al, 2003, Nestel et al, 2005 & Abdullah et al, 2015). Therefore, it is critical and urgent to understand better the association between dairy fats intake and risk of CVD comparing with other nutrients.

Data from Chapter II demonstrated that plasma total SFA after cheese treatment was significantly higher than after carbohydrate, MUFA and PUFA treatments, whereas there were no differences between butter, cheese and carbohydrate treatment. RBC total SFA after cheese, butter, carbohydrate and PUFA treatments were found to be not different from each other. Despite the fact that cheese and butter treatments significantly increased plasma palmitic acid levels; however, the effects were minor in RBC tissue concentrations. Particularly, RBC palmitic acid after cheese treatment was not different from all other treatments. Present results suggest that consuming two types of dairy fat, namely from cheese and butter can modulate circulating fatty acid profiles in a manner that increases plasma total SFA including palmitic acid, but have a minor effect on RBC tissue concentrations. As RBC fatty acid profile is considered to predict long-term changes of circulating fatty acid profiles. These findings can further question current dietary recommendations, whether SFA should be replaced by other nutrients and by how much. In addition, results from Chapter II also suggest that pentadecanoic acid, heptadecanoic acid and possibly myristic acid are potent biomarkers for indicating dairy fats intake. Changes of specific FA particular the odd-chain SFA from dairy, reflect an excellent means of establishing compliance level of study participants in human intervention studies.

It is also critical to understand the metabolism of fatty acid at a whole body level.

Endogenous *de novo* fatty acid synthesis is a crucial metabolic process that can modulate risk of several metabolic diseases such as obesity and CVD. Findings suggested that unsaturated fatty acids can inhibit the process of mammal's *de novo* fatty acid synthesis while improving fatty acid oxidation and whole body energy expenditure, whereas SFA may not (Wilson et al, 1990, Flick et al, 1977, Hausman et al, 2002, Jones et al, 1985 & Jones et al, 2008). However, results from Chapter III showed that consuming dairy fats does not modulate human *de novo* fatty acid synthesis compared with other nutrients such as MUFA, PUFA, and carbohydrate. Despite the fact that there were virtually no differences observed for *de novo* fatty acid synthesis across all treatments. However, *de novo* fatty acid synthesis was found to be negatively associated with plasma palmitic acid levels and positively associated with body fat mass. These findings suggest that the quality of fatty acids does not significantly modulate *de novo* fatty acid synthesis in humans for a duration of 28 days intervention; however, *de novo* fatty acid synthesis was found to be associated with body fat mass, which factors go beyond dietary fatty acids may contribute to this association.

#### **4.2 Limitation and future perspectives**

The randomized, crossover, full-feeding, controlled design was employed in the present study, which is believed to provide much more reliable findings than other types of intervention. The 28 days intervention design of the present study was sufficient to observe changes of plasma and RBC fatty acid profiles; however, may not be sufficient to detect changes of *de novo* fatty acid synthesis in response to dietary fats. On the other hand, factors which go beyond diet may also associate with *de novo* fatty acid synthesis, yet not been analyzed in the present study.

Future research is required.

### **4.3 Final conclusions**

In conclusion, consuming dairy fats, from namely cheese or butter can modulate circulating fatty acid profiles in a manner that increases plasma total SFA, myristic acid, pentadecanoic acid, palmitic acid and heptadecanoic acid levels; however, have a minor effect on RBC tissue concentrations. Consuming dairy fats for a duration of 28 days does not alter *de novo* fatty acid synthesis in humans compared with nutrients such as MUFA, PUFA, and carbohydrate.

#### 4.4 References

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# Appendices

## Appendix I: Ethics approval for study



Research Ethics - Bannatyne  
Office of the Vice-President (Research and International)

P126-770 Bannatyne Avenue  
Winnipeg, Manitoba  
Canada, R3E 0W3  
Telephone: [REDACTED]  
Fax: [REDACTED]

### BIOMEDICAL RESEARCH ETHICS BOARD (BREB) CERTIFICATE OF ANNUAL APPROVAL

<b>PRINCIPAL INVESTIGATOR:</b> Dr. P. Jones	<b>INSTITUTION/DEPARTMENT:</b> Richardson Centre for Functional Foods and Nutraceuticals/Food Sciences	<b>ETHICS #:</b> HS18188 (B2014:018)
<b>BREB MEETING DATE (If applicable):</b>	<b>APPROVAL DATE:</b> February 9, 2016	<b>EXPIRY DATE:</b> February 24, 2017
<b>STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (If applicable):</b>		

<b>PROTOCOL NUMBER:</b> 2013-251	<b>PROJECT OR PROTOCOL TITLE:</b> 2013-251 Integrated research program on dairy, dairy fat and cardiovascular health. Part 1: Impact of dairy fat compared with carbohydrates, mono and poly unsaturated fatty acids on plasma lipids and other cardiometabolic risk factors
<b>SPONSORING AGENCIES AND/OR COORDINATING GROUPS:</b> Laval University and Dairy Farmers of Canada	

<b>Submission Date of Investigator Documents:</b> January 28, 2016	<b>BREB Receipt Date of Documents:</b> January 29, 2016
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**REVIEW CATEGORY OF ANNUAL REVIEW:** Full Board Review  Delegated Review

**THE FOLLOWING AMENDMENT(S) and DOCUMENTS ARE APPROVED FOR USE:**

Document Name(if applicable)	Version(if applicable)	Date
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**Annual approval**

*Annual approval implies that the most recent **BREB approved** versions of the protocol, Investigator Brochures, advertisements, letters of initial contact or questionnaires, and recruitment methods, etc. are approved.*

**Consent and Assent Form(s):**

**CERTIFICATION**

The University of Manitoba (UM) Biomedical Research Board (BREB) has reviewed the annual study status report for the research study/project named on this **Certificate of Annual Approval** as per the category of review listed above and was found to be acceptable on ethical grounds for research involving human participants. Annual approval was granted by the Chair or Acting Chair, UM BREB, per the response to the conditions of approval outlined during the initial review (full board or delegated) of the annual study status report.

**BREB ATTESTATION**

The University of Manitoba (UM) Biomedical Research Board (BREB) is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement 2, and the applicable laws and regulations of Manitoba. In respect to clinical trials, the BREB complies with the membership requirements for Research Ethics Boards defined in

Division 5 of the Food and Drug Regulations of Canada and carries out its functions in a manner consistent with Good Clinical Practices.


#### QUALITY ASSURANCE

The University of Manitoba Research Quality Management Office may request to review research documentation from this research study/project to demonstrate compliance with this approved protocol and the University of Manitoba Policy on the Ethics of Research Involving Humans.

#### CONDITIONS OF APPROVAL:

1. The study is acceptable on scientific and ethical grounds for the ethics of human use only. ***For logistics of performing the study, approval must be sought from the relevant institution(s).***
2. This research study/project is to be conducted by the local principal investigator listed on this certificate of approval.
3. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to the research study/project, and for ensuring that the authorized research is carried out according to governing law.
4. **This approval is valid until the expiry date noted on this certificate of annual approval.** A **Bannatyne Campus Annual Study Status Report** must be submitted to the REB within 15-30 days of this expiry date.
5. Any changes of the protocol (including recruitment procedures, etc.), informed consent form(s) or documents must be reported to the BREB for consideration in advance of implementation of such changes on the **Bannatyne Campus Research Amendment Form**.
6. Adverse events and unanticipated problems must be reported to the REB as per Bannatyne Campus Research Boards Standard Operating procedures.
7. The UM BREB must be notified regarding discontinuation or study/project closure on the **Bannatyne Campus Final Study Status Report**.

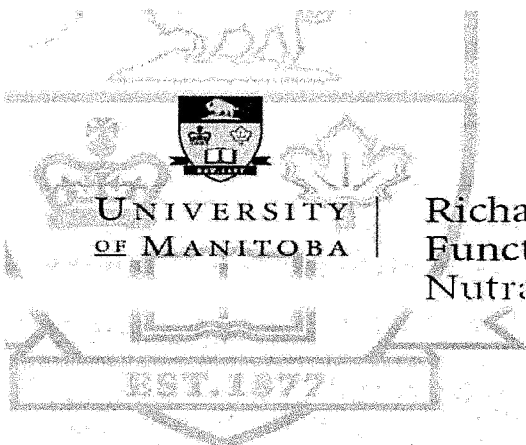
Sincerely,

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

Please quote the above Human Ethics Number on all correspondence.

Inquiries should be directed to the REB Secretary Telephone: (204) 789-3255/ Fax: (204) 789-3414

## Appendix II: Study forms



Richardson Centre for  
Functional Foods and  
Nutraceuticals

Room 106  
196 Innovation Drive  
Winnipeg, Manitoba  
Canada R3T 2N2  
Telephone (204) 474-8883  
Fax (204) 474-7552  
peter\_jones@umanitoba.ca

### RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

**Title of Study:** Integrated research program on dairy, dairy fat and cardiovascular health. Part 1: Impact of dairy fat compared with carbohydrates, mono and poly unsaturated fatty acids on plasma lipids and other cardiometabolic risk factors.

**Protocol #:** 2013-251

**Principal Investigator:**

Peter Jones, PhD  
Richardson Centre for Functional Foods and Nutraceuticals  
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Phone: [REDACTED]

**Co-Investigator:**

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**Sponsors:**

Benoit Lamarche,  
Institute on Nutrition and Functional Foods  
Laval University  
Pavillion des Services,  
2440 Hochelaga Blvd,  
Quebec, QC, G1V 0A6  
Phone [REDACTED]

Dairy Farmers of Canada  
1801 McGill College Avenue  
Suite # 700  
Montreal, QC H3A 2N4  
Phone: [REDACTED]



Title of the study: Integrated research program on dairy, dairy fat and cardiovascular health. Part 1: Impact of dairy fat compared with carbohydrates, mono and poly unsaturated fatty acids on plasma lipids and other cardiometabolic risk factors

You are being asked to participate in a Clinical Trial (a human research study). Please take your time to review this 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 before you make your decision. 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. The study doctor (and or/ institution) is (are) receiving professional fees and financial support to conduct this study.

**Purpose of Study**

This Clinical Trial is being conducted to study the effects of consumption of dairy fat compared with carbohydrates, mono and poly unsaturated fatty acids on plasma lipids and other risk factors for heart disease. You are being asked to take part in this study because you are aged between 18-65 yrs and you have a waist girth and blood triglycerides moderately elevated. A total of 70 (35 from Winnipeg area and 35 from Québec City area) participants will participate in this study.

This research is being done because market trends depicted by Agriculture and Agri-Food Canada suggest stagnation in cheese consumption, with potentially important impact on this key industry in Canada. This is in part due to the commonly accepted notion that saturated fat in the diet, of which cheese contributes significantly, increases the risk of heart disease. Yet, a rather large body of recent evidence suggests that although saturated fat may have been projected to be unhealthy its impact on the risk of heart disease may in fact be less important than originally thought. This concept that dairy fat increases the risk of heart attacks therefore needs to be revisited, and this is one of the key objectives of this proposed research program.

**Study procedures**

If you take part in this study, you will have the following tests and procedures:

**Screening:** If you agree to take part in this study, as part of a screening visit, you will be asked to have a fasting (nothing to eat or drink 12 hours before the test) blood sample of approximately two teaspoons taken to measure your blood fat levels. During screening, we will also measure your body weight, height, waist circumference and blood pressure as well as your record your age. In order to be eligible for the study, your age should be between 18 and 65 with waist circumference greater than 80 cm for women and greater than 94 cm for men, as well as with blood HDL-Cholesterol levels  $\leq 1.53$  mmol/L for women and  $\leq 1.34$  mmol/L for men. Individuals with a previous history of cardiovascular disease, type 2 diabetes, on cholesterol lowering or hypertension medications and smokers will not be eligible to participate. If you are eligible to participate based on your screening result you will begin the trial. Pregnancy tests will be performed for all pre-menopausal female participants at screening visits and at the beginning of each phase, if the test is positive at screening or during the study they will be asked to stop participation in the study. Any change in your health status at any point during the study needs to be reported to the study investigators.

**Dietary study:** The study will consist of 5 phases of 4 weeks each during which you will consume morning breakfast along with your assigned treatment under supervision. The five treatment phases will be interrupted by 4 weeks washout phases during which you follow your habitual diets. We will ask that you limit your consumption of alcohol and caffeinated beverages throughout the phases.



Title of the study: Integrated research program on dairy, dairy fat and cardiovascular health. Part 1: Impact of dairy fat compared with carbohydrates, mono and poly unsaturated fatty acids on plasma lipids and other cardiometabolic risk factors

**Dietary and physical activity assessment:** Before the beginning of the study, you must complete a food frequency questionnaire on the Web (duration of about 45 minutes) to assess the energy content (calories) and the proportions of protein, fat and carbohydrates (sugars) of your regular diet. Habits of physical activity will also be assessed using a standardized questionnaire.

**Blood samples:** At the screening visit, fasting blood sample of 2 teaspoons will be collected to check your blood counts, your lipid profile and to ensure the proper functioning of your liver, your kidneys and your thyroid gland. During days 1, 2 of each phase fasting blood samples of approximately 22 mL will be collected during each of these 2 days. During days 29 and 30 of each phase fasting blood samples of approximately 42 ml will be collected during each of these 2 days. These blood samples will be used to assess your lipid profile and other risk factors for heart disease. The total amount of blood collected for all samples in the study will be about 650 ml (about 2 ½ cups) spread over a period of about nine months. Generally a person can give up to 450 ml of blood every 2 months safely. The amount of blood collected in the context of this project is lower than that. However, you must refrain from donating blood during the study and for a period of two months following your participation in this project. Finally, you should refrain from vigorous physical activity 3 days before each blood sampling.

**Resting blood pressure:** Blood pressures at rest (following a 10-minute rest) will be taken early in the morning after a 12 hours fasting and will be repeated twice at an interval of three minutes. These measures will be taken at the first screening visit and at the beginning and at the end of each 4-weeks period.

**Anthropometric measurement:** Measures of your weight, your height (once), your waist and your hip circumference will be done at the screening visit and at the beginning and at the end of each 4-week period. Taking waist and hip circumference is done using a measuring tape and involves no pain. You will be weighed every day of the week during each 4-weeks period to monitor changes in your weight.

**Body composition measurement (DXA):** Body composition (fat mass and lean mass) will be measured with a procedure called dual energy x-ray absorptiometry (DXA). The examination takes place in a room specially designed for this test. A radiology technician or other person authorized to operate the device will perform the test. The test does not require any special preparation for the subject. You will be asked not to wear anything metal. The procedure takes about 15 minutes and the dose of radiation is very low (0.037 to 0.074 mrem according to the exposure time required to obtain results of high precision). The total exposure in this project with 5 measures of body composition with DXA will be a maximum of 0.185 mrem, which is 54 times less than a dental x-ray exposure which is estimated at 10 mrem. Exposure to cosmic rays during a 6 hours flight from Montreal to Vancouver is estimated at 3 mrem. The exposure in this project level is therefore quite safe.

### **Experimental diets**

The 5 phases of treatments will include:

- 1- A diet containing saturated fat from cheese (13% of calories as saturated fat);
- 2- A diet containing saturated fat from butter (13% of calories as saturated fat);
- 3- A carbohydrate-rich diet low in saturated fat (6% of calories from saturated fat);

Title of the study: Integrated research program on dairy, dairy fat and cardiovascular health. Part 1: Impact of dairy fat compared with carbohydrates, mono and poly unsaturated fatty acids on plasma lipids and other cardiometabolic risk factors

- 4- A diet rich in monounsaturated fat, mainly from olive oil, and low in saturated fat (21% of calories from monounsaturated fat, 6% saturated fat);
- 5- A diet rich in polyunsaturated fat, largely from sunflower oil, and low in saturated fat (12% of calories as polyunsaturated fat, 6% saturated fat).

The nutrient composition of the diets will be as follows:

Diets					
	CHEESE	BUTTER	CHO	MUFA	PUFA
Protein, %	15	15	15	15	15
CHO, %	53	53	60	53	53
FAT, %	32	32	25	32	32
SFA, %	13	13	6	6	6
MUFA, %	14	14	14	21	14
PUFA, %	5	5	5	5	12

CHO-Carbohydrates; SFA-Saturated fatty acids; MUFA-Monounsaturated fatty acids; PUFA-Polyunsaturated fatty acids.

The experimental diets were based on the Canada's Food Guide recommendations. No known risk of short term deficiency or excess in energy, protein, fat or carbohydrate is present with the experimental diets. Two of the 5 experimental diets contained 13% of calories from saturated fat, which is slightly above the average consumption for Canadian adults, which is about 11%. However, for 3 of the 5 experimental diets, the relative content of saturated fat is 6% of calories. So on average over the experimental period of 20 weeks, your average intake of saturated fat is 8.8%, which meets the target of the Canadian recommendations.

This study is with a double blinded design which means neither you nor the clinical staff will know which diets you will be receiving. In an emergency, this information will be made available. You will receive all 5 diets. These blood samples will be obtained for assessment of blood fat levels and fat metabolism. Each blood test will take approximately 5 minutes.

For the purpose of subsequent analysis, we will store plasma and serum samples. At the end of the study, your records will be stored in accordance with Health Canada's regulations for 25 years-at RCFFN. During the course of the study, processed blood will be stored at the RCFFN in -80°C freezers, for a maximum of 5 years from the end of the study where possible. At this point the investigators will re-apply for a further extension of storage to accommodate new analysis indicated at that time. In the absence of such an application, all samples will be destroyed by autoclaving. During the storage period if you should change your mind about your samples being stored you have the opportunity to withdraw your consent. Simply call the centre at [REDACTED] or any of the investigators listed on page 1 of this consent and inform them that you wish to withdraw your consent. The stored blood will be available for re-analysis of samples if values are called into question. In which case we may attempt to contact you by phone or letter requesting that you attend an information session where the new information will be discussed.

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Participation in the study will be for 36 weeks. The researcher may decide to take you off this study if it is in the participant's medical best interest, participant's condition worsens, failure to follow the study protocol. You can stop participating at any time. However, if you decide to stop participating in the study, we encourage you to talk to the study staff and your regular doctor first.

There are no serious health consequences of sudden withdrawal from the study for you. Your participation in this research project is entirely on a volunteer basis. You can refuse to participate or you can interrupt your participation anytime, throughout the study period, without any penalty or loss of benefits to which you would otherwise be entitled. Participants will receive a sealed and confidential letter which states their individual results of their blood tests, global physical health and dietary evaluation along with the mean values obtained from the entire study population. The letter will be sent by the principal investigator at RCFFN to the mailing address on the personal information form that participants fill out prior to enrolment to the study.

### **Risks and Discomforts**

As with any clinical trial, there may be as yet unknown or unforeseen risks of taking part in the study. 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. In case you feel any discomfort during the experimental trial a physician, Dr. Shahrokh Nejad Ghaffar, will be available to contact at any time. Dr. Shahrokh Nejad Ghaffar can be reached at [REDACTED]

### **Benefits**

There may or may not be direct medical benefit to you from participating in this study. We hope the information learned from this study will benefit other participants with increased risk for cardiovascular disease in the future. In addition to the above, you will also receive your results when they become available.

### **Costs**

All clinic and professional fees, diagnostic and laboratory tests which will be performed as part of this study are provided at no cost to you. There will be no cost for the study treatment 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 \$1500 at completion of this study for your time and inconvenience of the study schedule. This amount will be divided into 5 portions. You will receive \$200 after the completion of each phase until the end of phase 4 and \$450 after the completion of phase 5. You would also get an additional \$50 per phase for fecal collection. If you withdraw early from the study, you will receive an appropriate pro-rated fraction of this amount.



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### **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.

### **Confidentiality**

Information gathered in this research study may be published or presented in public forums, however your name and other identifying information will not be used or revealed. Medical records that contain your identity will be treated as confidential in accordance with the Personal Health Information Act of Manitoba. Despite efforts to keep your personal information confidential, absolute confidentiality cannot be guaranteed. Your personal information may be disclosed if required by law. All study documents related to you will bear only your assigned patient number (or code) and /or initials.

All data pertaining to the measurements that will be done in this multicenter study will be shared with the partner institution (INAF, Laval University). These data will be entered into the private website (<http://inaf.fsaa.ulaval.ca/fani/index.php?langue=en>) developed by Dr. Benoit Lamarche and team at the Laval University protected with username and password. Study coordinators at RCFFN and INAF only will have access to this website. Your name and personal information (name, address, and phone number) will be kept confidential and won't be transmitted to the partner or entered in the above mentioned private website. Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as: Study coordinator of INAF will be accessing research study records of participants. The University of Manitoba Biomedical Research Ethics Board may review research-related records for quality assurance purposes.

All records will be kept in a locked secure area and only those persons identified will have access to these records. If any of your medical/research records need to be copied to any of the above, your name and all identifying information will be removed. No information revealing any personal information such as your name, address or telephone number will leave the Richardson Centre for Functional Foods and Nutraceuticals. With your permission your Family Physician (GP) will be notified about your participation in this study.

The study is registered on a publicly available registry databank at [clinicaltrials.gov](http://clinicaltrials.gov). ClinicalTrials.gov is a website that provides information about federally and privately supported clinical trials. A description of this clinical trial will be available on <http://ClinicalTrials.gov>. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

### **Voluntary Participation/Withdrawal From the Study**

Your decision to take part in this study is voluntary. You may refuse to participate or you may withdraw from the study at any time. Your decision not to participate or to withdraw from the study will not affect your other medical care at this site. If your study doctor feels that it is in your best interest to withdraw you from the study, your study doctor will remove you without your consent. We will tell you about any new information that may affect your health, welfare, or willingness to stay in this study.

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If you decide to participate, you will agree to co-operate fully with the study visit schedule, and will follow the study staff's instructions. If you are an employee of University of Manitoba, be sure that your performance evaluation will not be affected by your decision not to participate. Should you wish to withdraw your participation from the study, you must inform the study coordinators so that your file can be officially closed.

### **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. Shahrokh Nejad Ghaffar at [REDACTED] or go to your nearest emergency room to receive necessary medical treatment. You are not waiving any of your legal rights by signing this consent form or releasing the investigator(s) or the sponsor from their legal and professional responsibilities. If any health abnormalities are identified in the clinical tests conducted during this experiment, Dr. Shahrokh Nejad Ghaffar 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 participant. 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 listed in page 1. For questions about your rights as a research participant, you may contact The University of Manitoba Biomedical Research Ethics Board at [REDACTED]. Do not sign this consent form unless you have had a chance to ask questions and have received satisfactory answers to all of your questions.

### **Statement of Consent**

I have read this consent form. I have had the opportunity to discuss this research study with Peter Jones and or his/her study staff. I have had my questions answered by them in language I understand. The risks and benefits have been explained to me. I believe that I have not been unduly influenced by any study team member to participate in the research study by any statement or implied statements. Any relationship (such as employee, student or family member) I may have with the study team has not affected my decision to participate. I understand that I will be given a copy of this consent form after signing it. I understand that my participation in this clinical trial is voluntary and that I may choose to withdraw at any time. I freely agree to participate in this research study.

I understand that information regarding my personal identity will be kept confidential, but that confidentiality is not guaranteed. I authorize the inspection of my medical records by Benoit Lamarche and his team at Laval University, Dairy Farmers of Canada, the Food and Drug Administration, the Health Protection Branch, government agencies in other countries, and The University of Manitoba Biomedical Research Ethics Board. By signing this consent form, I have not waived any of the legal rights that I have as a participant in a research study.

Title of the study: Integrated research program on dairy, dairy fat and cardiovascular health. Part 1: Impact of dairy fat compared with carbohydrates, mono and poly unsaturated fatty acids on plasma lipids and other cardiometabolic risk factors

I agree to being contacted in relation to this study. Yes  No

I agree to my family physician being notified of my participation in this study. Yes  No

I agree to being contacted for future studies at the RCFN. Yes  No

I agree to participate in the fecal sample collection Yes  No

Participant signature \_\_\_\_\_

Date \_\_\_\_\_  
(day/month/year)

Participant printed name: \_\_\_\_\_

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has knowingly given their consent

Printed Name: \_\_\_\_\_ Date \_\_\_\_\_  
(day/month/year)

Signature: \_\_\_\_\_

Role in the study: \_\_\_\_\_





UNIVERSITY  
OF MANITOBA

Richardson Centre for  
Functional Foods and  
Nutraceuticals

Room 106  
196 Innovation Drive  
Winnipeg, Manitoba  
Canada R3T 2N2  
Telephone (204) 474-8883  
Fax (204) 474-7552  
peter\_jones@umanitoba.ca

**PARTICIPANT INFORMATION AND CONSENT FORM**

**For permission to be contacted for future research at the Richardson Centre for Functional Foods and Nutraceuticals**

**Principal Investigator:** Peter J.H. Jones, PhD  
Richardson Centre for Functional Foods and Nutraceuticals  
196 Innovation Drive  
University of Manitoba, SmartPark  
Winnipeg, Manitoba R3T 6C5  
Phone: [REDACTED]

You are being asked for permission to be contacted in the future for participation in research studies. Please take your time to review this consent form and discuss any questions you may have. You are free to discuss this form with your friends, family and others before you make your decision.

If you agree to be contacted in the future for research purposes, information about you will be entered into an electronic database. The database will be maintained by Dr. Peter Jones, (Principal Investigator) and Julia Rempel (Clinical Coordinator) at the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN). You are only agreeing to be contacted about participating. For each study you will be given additional information including a consent form specific to that study.

The Database will have the following information about you: name, phone number, mailing address and email address.

Confidentiality of your information will be maintained in the following manner:  
Only the Principal Investigator and Clinical Coordinators at the RCFFN will have access to the electronic database which is on a password protected computer in a locked office. No outside clinics or institutions will have access to your information. Your contact information will be kept for 10 years. After 10 years your information will be destroyed.

This consent form and the information in the database may be inspected by a University of Manitoba Research Ethics Board to ensure that your information is being collected and maintained in an ethical manner.

Your decision to allow your information to be in the database is completely voluntary. While there may be no benefit to you, your information will help researchers to find individuals that may be interested in participating in a research study. If you change your mind after agreeing to this, your information can be removed from the database. You will not be penalized in any way if you refuse to participate, or if change your mind and ask that your information be removed.

If you have any questions about this database, please contact:

Dr. Peter Jones at [REDACTED] or Julia Rempel at [REDACTED]

If you no longer want to be part of the research database, please contact:

Julia Rempel at [REDACTED]

If you have questions about your rights as a research participant, you may contact The University of Manitoba, Bannatyne Campus Research Ethics Board Office at [REDACTED]

Do not sign this consent form unless you have had a chance to ask questions and have received satisfactory answers to all of your questions.

Statement of Consent

I have read this consent form. I have had the opportunity to ask questions and discuss what is involved. I understand that my personal information will be kept confidential. By signing this consent form, I have not waived any of my legal rights.

Participant signature \_\_\_\_\_ Date \_\_\_\_\_

Participant printed name: \_\_\_\_\_

I, the undersigned, have fully explained the relevant details of this research to the participant named above and believe that the participant has understood and has knowingly given their consent

Printed Name: \_\_\_\_\_ Date \_\_\_\_\_

Signature: \_\_\_\_\_

Role: \_\_\_\_\_

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

**General information**  
PROJECT HDL #2013-251

Name : \_\_\_\_\_ Family Name: \_\_\_\_\_

Gender :  W  M

Date of birth : \_\_\_/\_\_\_/\_\_\_ 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 at lunchtime:  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?

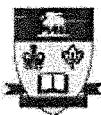
Yes  No

If yes, precise: \_\_\_\_\_

COMMENTS

\_\_\_\_\_

\_\_\_\_\_



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

**Medical Questionnaire  
SCREENING S1**

Sex :  M  W

Date of birth: \_\_\_\_\_

Age: \_\_\_\_\_

**BLOOD PRESSURE AT REST (AFTER 10 MINUTES OF REST)**

ARM:  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) = \_\_\_\_\_ Avg. Diastolic BP (mmHg) = \_\_\_\_\_

Heart rate / min = HR(1): \_\_\_\_\_ HR(2): \_\_\_\_\_ HR(3): \_\_\_\_\_ HR(mean): \_\_\_\_\_

**ANTHROPOMETRIC MEASUREMENTS**

Body weight (kg): \_\_\_\_\_ Height (m): \_\_\_\_\_ BMI (kg/m<sup>2</sup>): \_\_\_\_\_

Waist girth (cm) : WG(1): \_\_\_\_\_ WG(2): \_\_\_\_\_ WG(3): \_\_\_\_\_

Hip girth (cm) : HG(1): \_\_\_\_\_ HG(2): \_\_\_\_\_ HG(3): \_\_\_\_\_

Completed by: \_\_\_\_\_

### ETHNICITY

- Caucasian (eg North America, Europe, Middle East, North Africa, etc.)
- African and Afro-American (eg Jamaica, Caribbean, Niger, Haiti, etc.)
- Autochthon
- Inuit (eg Eskimo)
- Asian (eg Far East, Southeast Asia, Cambodia, China, etc.)
- Hispanic (eg Mexico, Cuba, Dominican Republic, etc.)
- Other: \_\_\_\_\_

### BLOOD SAMPLES: CHECKLIST

Did you eat or drink anything except water in the last 12 hours?

Yes       NO

Have you consumed alcohol or a product containing alcohol in the last 48 hours?

Yes       NO

In the last 24 hours, have you done intensive exercise?

Yes       NO

**BLOOD SAMPLES:**

LEFT ARM

RIGHT ARM

**BLOOD SAMPLES:**

Yes

NO

**EXTRA BLOOD SAMPLE (Keep frozen)**

Yes

NO

**Nurse's initials:** \_\_\_\_\_

**Comments:**

\_\_\_\_\_  
\_\_\_\_\_



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

**Medical Questionnaire D1-D29**

Diet 1       Diet 2       Diet 3       Diet 4       Diet 5

**BLOOD PRESSURE AT REST (AFTER 10 MINUTES OF REST)**

ARM :                       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) = \_\_\_\_\_ Avg. Diastolic BP (mmHg) = \_\_\_\_\_

Heart rate / min = HR(1): \_\_\_\_\_ HR(2): \_\_\_\_\_ HR(3): \_\_\_\_\_ HR(mean): \_\_\_\_\_

Side effects questionnaires completed:      Yes       No

**ANTHROPOMETRIC MEASUREMENTS**

Body weight (kg): \_\_\_\_\_

Waist girth (cm) : WG(1): \_\_\_\_\_ WG(2): \_\_\_\_\_ WG(3): \_\_\_\_\_

Hip girth (cm) : HG(1): \_\_\_\_\_ HG(2): \_\_\_\_\_ HG(3): \_\_\_\_\_

Completed by: \_\_\_\_\_

Date of your last periods (1<sup>st</sup> day) : \_\_\_\_\_

Not applicable:

**BLOOD SAMPLES: CHECKLIST**

Did you eat or drink anything except water in the last 12 hours?

Yes  NO

Have you consumed alcohol or a product containing alcohol in the last 48 hours?

Yes  NO

In the last 24 hours, have you done intensive exercise?

Yes  NO

**BLOOD SAMPLES:**

LEFT ARM

RIGHT ARM

**BLOOD SAMPLES:**

Yes

NO

**EXTRA BLOOD SAMPLE (Keep frozen)**

Yes

NO

**Nurse's initials:** \_\_\_\_\_

**For visit D1 of diet 2, diet 3, diet 4, diet 5 :**

**Washout duration before this diet:** \_\_\_\_\_ days

Not applicable (D1 of the first diet)

Not applicable (visit D29)

**STABLE ISOTOPE TRACER INTAKE: CHECKLIST**

**Intake of <sup>13</sup>C-palmitate on D29**

Yes

NO

**Intake of Deuterium on D29**

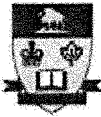
Yes

NO

Not applicable (visit D1)

**COMMENTS**

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_



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

**Medical Questionnaire D2-D30**

Diet 1     Diet 2     Diet 3     Diet 4     Diet 5

**ANTHROPOMETRIC MEASUREMENTS**

Body weight (kg): \_\_\_\_\_

**BLOOD SAMPLES : CHECKLIST**

Did you eat or drink anything except water in the last 12 hours?  Yes     NO

Have you consumed alcohol or a product containing alcohol in the last 48 hours?  Yes     NO

In the last 24 hours, have you done intensive exercise?  Yes     NO

**BLOOD SAMPLES:**                       LEFT ARM                       RIGHT ARM

**BLOOD SAMPLES:**                       Yes                       NO

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

Nurse's initials: \_\_\_\_\_

For visit D30 of all diets:

Number of days on the diet: \_\_\_\_\_ days  
 Not applicable (visit D2)

**CHANGE IN MEDICATION**                      Yes                       No

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







DATE OF OBSERVATION			INITIALS OF SUBJECT		# SUBJET(CODIFICATION)	
DAY	MONTH	YEAR	NAME	LAST NAME	HDL	

**QUESTIONNAIRE ON SIDE EFFECTS-INTENSITY**

Diet 1       Diet 2       Diet 3       Diet 4       Diet 5

Day 1                       Day 30

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 <sup>0</sup>	Mild <sup>1</sup>	Moderate <sup>2</sup>	Severe <sup>3</sup>
1. Headache	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
2. Anxiety	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
3. Fatigue / exhaustion	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
4. Lack of energy	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
5. Tend to become exhausted quickly	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
6. Decreased appetite	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7. Increased appetite	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
8. Hiccup	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
9. Nausea	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
10. Vomiting	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
11. Indigestion	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
12. Stomach or abdominal pain	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
13. Constipation	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
14. Diarrhea	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
15. Flatulence	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
16. Abdominal bloating	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
17. Palpitations	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
18. Balance disorders	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
19. Decreased ability to concentrate	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
20. Flushing	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
21. Feeling cold	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
22. Joint or members pain	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
23. Numbness, burning or itching (feet/hands)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
24. Dark or depressing thoughts	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
25. Other	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>



DATE OF OBSERVATION			INITIALS OF SUBJECT		# SUBJET(CODIFICATION)	
DAY	MONTH	YEAR	NAME	LAST NAME	HDL	

**QUESTIONNAIRE ON SIDE EFFECTS-FREQUENCY**

Diet 1       Diet 2       Diet 3       Diet 4       Diet 5

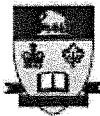
Day 1                       Day 30

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 <sup>0</sup>	Rarely <sup>1</sup>	Sometimes <sup>2</sup>	Often <sup>3</sup>
1. Headache	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
2. Anxiety	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
3. Fatigue / exhaustion	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
4. Lack of energy	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
5. Tend to become exhausted quickly	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
6. Decreased appetite	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7. Increased appetite	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
8. Hiccup	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
9. Nausea	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
10. Vomiting	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
11. Indigestion	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
12. Stomach or abdominal pain	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
13. Constipation	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
14. Diarrhea	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
15. Flatulence	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
16. Abdominal bloating	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
17. Palpitations	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
18. Balance disorders	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
19. Decreased ability to concentrate	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
20. Flushing	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
21. Feeling cold	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
22. Joint or members pain	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
23. Numbness, burning or itching (feet/hands)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
24. Dark or depressing thoughts	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
25. Other	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>



DAY	OBSERVATION DATE			SUBJECT'S INITIAL		# SUBJECT (CODIFICATION)	
	DAY	MONTH	YEAR	FIRST NAME	LAST NAME	HDL	
D30							

Diet 1     Diet 2     Diet 3     Diet 4     Diet 5

**Short osteodensitometry questionnaire**

Sex:  Women  Men

1. Have you already had surgical intervention that needed screw, metal plate or prosthesis? Precise : _____	<input type="checkbox"/> Yes <input type="checkbox"/> No
2. Do you have any "piercings"?	<input type="checkbox"/> Yes <input type="checkbox"/> No
3. Have you ever had a scoliosis diagnosis?	<input type="checkbox"/> Yes <input type="checkbox"/> No
4. In the past 2 weeks, have you had an exam involving any radioactive medicine? (nuclear medicine, barium) Why? _____ <i>If yes, don't do the test</i>	<input type="checkbox"/> Yes <input type="checkbox"/> No
5. If you are woman, do you have the possibility of being pregnant? <i>If yes, don't do the test</i>	<input type="checkbox"/> Yes <input type="checkbox"/> No
6. Would you like a pregnancy test? If No, Why? _____	<input type="checkbox"/> Yes <input type="checkbox"/> No

Comments:

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**Requisition for DXA Scan**

**Study name:** Integrated research program on dairy, dairy fat and cardiovascular health. Part 1: Impact of dairy fat compared with carbohydrates, mono and poly unsaturated fatty acids on plasma lipids and other cardiometabolic risk factors

**Ethics #:**           B2014:018          

**Scans Ordered by:**

Name:

Phone Number:

Account to be billed:

**Primary Investigator:**

**Name:**           Dr. Peter Jones           **Signature:** \_\_\_\_\_

**Physician co-signer:**

**Name:**           Dr. Shahrokh Nejad Ghaffar           **Signature:** \_\_\_\_\_

**Subjects to be studied:**               Human                      Animal (Specify)          

**Subjects Name:** \_\_\_\_\_

**Subjects Study ID #:** \_\_\_\_\_

**Measurements:**             Length/Height \_\_\_\_\_ cm     DOB(DD/MM/YY):           /          /            
  Weight \_\_\_\_\_ kg

**Total Number of Scans Approved in REB protocol for each individual:**           5          

**Pregnancy Test conducted**               Yes                     No                     Not Applicable          

**Scans Required:**

**Scan ID#**  
\_\_\_\_\_  Whole Body     \_\_\_\_\_ Body Part (Specify) \_\_\_\_\_  
\_\_\_\_\_            Infant     \_\_\_\_\_ Pediatric     \_\_\_\_\_ Adult     \_\_\_\_\_ Small Animal (circle one)

**DATE OF Scan (DD/MM/YY) #**            :           /          /               **DATE OF Scan (DD/MM/YY) #**            :           /          /            
**DATE OF Scan (DD/MM/YY) #**            :           /          /               **DATE OF Scan (DD/MM/YY) #**            :           /          /            
**DATE OF Scan (DD/MM/YY) #**            :           /          /               **DATE OF Scan (DD/MM/YY) #**            :           /          /          

**Required Format: (check all the apply)**                Hard Copy                 CD-ROM

Charge Information- Office Use Only

## **Fatty acid Methylation method**

### **Chemicals needed:**

- Internal standard 1mg/mL in chloroform
- Methanol
- Toluene
- Acetyl chloride
- K<sub>2</sub>CO<sub>3</sub> 6% (Potassium Carbonate Anhydrous). Take 6g of K<sub>2</sub>CO<sub>3</sub> + 100ml of DDH<sub>2</sub>O

### **Procedure**

(Vortex the samples first)

1. Weight 0.5g of RBC in a glass tube.
2. Add 1.6ml methanol and vortex for 20s.
3. Add 75ul of internal standard and vortex for 5s.
4. Add 400ul of toluene and vortex for 20s.
5. While vortexing, slowly add 200ul of acetyl chloride in the tube.
6. Flash the tube under nitrogen for 10s and cap tightly.
7. Vortex tubes for 10s.
8. Heat tubes in dry bath at 80 C for 1hour.
9. Let cool.
10. Add 5ml K<sub>2</sub>CO<sub>3</sub> 6% and vortex for 20s (6g K<sub>2</sub>CO<sub>3</sub> + 100ml DDH<sub>2</sub>O).
11. Centrifuge tubes at 2500rpm for 5min.
12. Transfer top layer in a GC vial with insert. Cap to seal for GC analysis.

**END OF DOCUMENT**