Genetic variants influence the response of body composition and insulin resistance to dietary monounsaturated fatty acids consumption

by

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ABSTRACT

Obesity and insulin resistance (IR) control is a requisite for reducing the risk of several non-communicable disease states. Although monounsaturated fatty acids (MUFA) advantageously affect obesity and IR risk, inter-individual variations in the responses of obesity and IR to dietary MUFA have been identified. Single-nucleotide polymorphisms (SNPs) profoundly influence such responsiveness; however, the effects of the interactions between dietary MUFA and genetic factors on obesity and IR have been scarcely tested in intervention studies. The objective of the present research was to investigate the impact of genetic variability on responsiveness of body fat mass and IR measure to a high level of dietary MUFA. In a randomized, crossover, controlled-feeding trial, 124 adults with abdominal obesity consumed one of three oils (20% of total energy) each for six weeks, separated by a four-12 week washout. Oils included two-high-MUFA oils, conventional canola and high-oleic canola, and a low-MUFA high-saturated fatty acid (CONTROL) oil blend. In this study, no significant differences were observed in changes in body weight, fat mass, or measures of glycemic control and IR following the consumption of any of the three treatments. However, results reveal that SNPs within obesity-related genes contributed to shaping the degree of body fat response to dietary MUFA. The consumption of high-MUFA diets induced significant reductions in various regions of fat mass in the *LPL* rs13702-CC, *PPARα* rs6008259-AA, and *ADIPOQ* rs266729-GG carriers compared to the opposing genotypes. Further, the CONTROL diet promoted fat mass reductions in participants possessing the *FTO* rs9939609-A, *APOE4*, *ADRB2* rs1042714-GG, and *LIPC* rs6083-GG variants, but not in individuals carrying the other genotypes. SNPs within IR-associated genes were found to modify the response of IR measure to dietary fatty acid modification; improvement in insulin sensitivity measures was observed upon consumption of the CONTROL

diet compared to the high-MUFA diets in the homozygotes of either the *FABP1* rs2241883-CC or the *IRS1* rs7578326-GG genotype compared to corresponding genotypes. These findings advance knowledge of the inherited basis by which alleviation of obesity and IR may be achieved in response to dietary fatty acid modification, hence launching an important step towards an era of personalized nutrition.

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ABBREVIATIONS

11β-HSD1	11β-hydroxysteroid dehydrogenase type-1
ACOX1	Acyl-CoA oxidase-1
ADRB2	Adrenoceptor Beta-2
ADIPOQ	Adiponectin
APOE	Apolipoprotein E
BMI	Body mass index
CD36	Cluster of differentiation-36
C/EBP-α	CCAAT-enhancer binding protein-α
COMIT	Canola oil multi-center intervention trial
CPT1A	Carnitine palmityl transferase-1A
CPT1B	Carnitine palmityl transferase-1B
Ct	Cycle threshold
DGAT1	Diacylglycerol O-acyltransferase
DXA	Dual energy X-ray absorptiometry
FA	Fatty acids
FABP1	Liver fatty acid binding protein
FABP4	Fatty acid binding protein-4
FTO	Fat mass and obesity-associated gene

HOCO	High-oleic canola oil
HOMA-β	Homeostasis model assessment of beta-cell function
HOMA-IR	Homeostasis model assessment of insulin resistance
IR	Insulin resistance
IRS1	Insulin receptor substrate-1
LEP	Leptin
LIPA	Lipase A, lysosomal acid
LIPC	Hepatic lipase
LIPE	Hormone-sensitive lipase
LIPF	Gastric lipase
LIPG	Endothelial Lipase
LPL	Lipoprotein lipase
MCFA	Medium-chain fatty acid
MCT	Medium-chain triglyceride
MET	Metabolic equivalents
MUFA	Monounsaturated fatty acid
n-3 PUFA	Omega-3 polyunsaturated fatty acid
n-6 PUFA	Omega-6 polyunsaturated fatty acid
PLIN1	Perilipin-1

PPARα	Peroxisome proliferator activated receptor-alpha
PPARδ	Peroxisome proliferator activated receptor-delta
PPARγ	Peroxisome proliferator activated receptor-gamma
PPARGC1A	Peroxisome proliferator-activated receptor gamma, coactivator-1 alpha
PPARGC1B	Peroxisome proliferator-activated receptor gamma, coactivator-1 beta
PRKAA2	Protein kinase, AMP activated, alpha catalytic subunit
PUFA	Polyunsaturated fatty acid
RCFFN	Richardson Centre for Functional Foods and Nutraceuticals
RCO	Regular canola oil
RETN	Resistin
SCAT	Subcutaneous adipose tissue
SCD	Stearoyl-CoA desaturase
SCD1	Stearoyl-CoA desaturase-1
SCD5	Stearoyl-CoA desaturase-5
SFA	Saturated fatty acid
SNP	Single nucleotide polymorphism
SLC27A1	Solute carrier family 27, member-6
SREBF1	Sterol regulatory element-binding transcription factor-1
SREBP1	Sterol regulatory element binding protein-1

TCF7L2	Transcription factor 7-like-2
UCP2	Uncoupling protein-2
VAT	Visceral adipose tissue
WC	Waist circumference
WHR	Waist-to-hip ratio

CHAPTER I

GENERAL INTRODUCTION

1.1 Introduction

The global prevalence of obesity is growing rapidly. In 2016, World Health Organization (WHO) global estimates suggest 39% of adults aged 18 years and over were overweight, and 13% were obese (1). A trend analysis showed that the prevalence of adult obesity in Canada, especially excessive obesity, continues to rise, whereas the prevalence of normal weight Canadian adult is steadily decreasing (2). The estimate indicates that half of the Canadian provinces will have more overweight or obese adults than normal weight adults by 2019 (2). Obesity can negatively affect the quality of life and is a major risk factor for cardiovascular disease, insulin resistance (IR), type 2 diabetes, and cancer (2, 3). The success rate of obesity control regimens is not always satisfactory, which can be attributed to the fact that obesity is a complex trait that is influenced by several determinants including diet, environment, and genetics (4). An effective obesity treatment and prevention strategy must contemplate the modifiable and heritable factors to achieve an optimal outcome.

1.1.1 Pathogenic obesity

Although obesity is a leading risk factor for various chronic diseases and metabolic abnormalities, the influence of obesity on health is greatly determined by the distribution pattern of body fat rather than only the level of fat accumulation (5, 6). Body fat accumulates in two main regions; abdominal (android) and peripheral (gynoid) (7, 8). Abdominal obesity is associated with increased risks for chronic diseases, cardio-metabolic disturbances, and IR compared to peripheral obesity (7-10). For instance, a two percent increase in the risk of future

cardiovascular disease was associated with one centimeter increase in waist circumference (8). Wiklund et al. have reported that an increase of less than one kilogram of android fat corresponded to an elevation in cardiovascular disease risk factors from zero to at least three factors (10). However, not all adipocytes in the android region induce the same degree of pathogenesis.

Two main types of fat depot have been recognized in the android region, namely subcutaneous adipose tissue (SCAT) and visceral adipose tissue (VAT) (11). SCAT is the adipocyte that lies just beneath the skin, while adipocytes that accumulate around the vital organ in the abdominal cavity is known as VAT. Studies have shown strong and independent associations between VAT depot, rather than SCAT, and risks for chronic diseases and metabolic abnormalities (7, 11-16). The pathogenicity of VAT can be attributed to numerous VAT-specific features including containing larger number of inflammatory and immune cells, enclosing more androgen and glucocorticoid receptors, having more lipolytic activity, producing a variety of pathogenic adipokines, and draining large amounts of free fatty acids (FAs) and adipokines directly into liver through the portal vein (11, 17). Thus, quantification of total and regional fat mass and discrimination between different fat depots can provide a better understanding of the pathogenesis of obesity and therefore, offer better insight into the risk of metabolic abnormalities including IR and disrupted glycemic control.

1.1.2 Insulin resistance

IR is the loss of optimal biological response to insulin at the insulin-sensitive tissue. Accumulation of fat, particularly visceral fat (18), promotes oversupply of lipid to insulin-sensitive tissue, which may increase lipid accumulation in muscle, liver, and other non-adipose tissue, thus interrupts normal insulin-mediated glucose uptake (19, 20). Furthermore, obesity, in

addition to being a risk factor for IR, can be exacerbated, along with obesity-related metabolic abnormalities, by IR (20-22). Recent data analysis from three randomized trials of overweight and obese participants showed that elevated baseline fasting plasma glucose concentrations, possibly reflecting insulin resistance, may reduce the effectiveness of a weight loss dietary intervention (23). Given the intricate relationship between obesity and IR, an effective intervention to prevent/restore insulin sensitivity should also focus on obesity reduction. The evidence underscores the role of dietary FA modification in ameliorating IR and fat accumulation (24-26).

1.1.3 Dietary monounsaturated fatty acid and obesity

Modifying body fat accumulation and distribution can reduce disease risk and improve metabolic profile. Different dietary FAs vary in their obesity-inducing effect (27, 28). Saturated FA (SFA) and trans-fat intakes have been linked to obesity, specifically abdominal fat deposition (29, 30). Controversy exists regarding the beneficial effects of polyunsaturated FA (PUFA) on weight loss and maintenance (31, 32) as well as on cardiovascular complications (33, 34). On the other hand, dietary monounsaturated FA (MUFA) has been gaining a particular scientific interest regarding its advantageous effects on glycemic control, cardiovascular health, as well as obesity-protective effects (25, 31, 35, 36).

An increasing body of evidence supports the favorable effect of MUFA on body composition (25, 37-39). Adherence to a diet rich in MUFA was reported to induce favorable effects on body weight, abdominal obesity, and fat mass (31, 40-42). A randomized, cross-over, *ad libitum* feeding study found that a diet containing ~ 23% MUFA and ~ 11% SFA of total energy induced small but significant reductions in body weight and fat mass compared to a high-SFA diet (~ 24% SFA and ~ 13% MUFA of total energy) in overweight and obese men (37).

Additionally, a diet rich in MUFA was shown to ameliorate pathogenic obesity by reducing abdominal fat accumulation (25, 43), and was suggested to shift fat storage toward SCAT, rather than VAT (38, 39). A randomized, crossover, controlled study found a tendency of a four-week high-oleic acid oil-rich diet to suppress android-to-gynoid fat ratio compared to a flaxseed oil-rich diet (27). The Canola Oil Multicenter Intervention Trial I (COMIT I) is a previous randomized, controlled, crossover trial that was conducted by our group and assessed the actions of different dietary unsaturated FA oil/oil blend on body composition (44). The authors reported that conventional canola oil and high-oleic acid canola oil (MUFA rich) prompted significant reductions in android fat mass, especially, in men compared to a PUFA rich oil blend.

These beneficial effects of MUFA consumption might be attributed to the increased propensity of MUFA to be oxidized rather than stored in adipocytes, as indicated by a considerable body of evidence (28, 45-47). Also, MUFA consumption was reported to increase energy expenditure and diet-induced thermogenesis compared to SFA consumption (46, 48-50). Kien et al. reported an additional attractive effect of high-MUFA consumption on elevating physical activity levels compared to a high-palmitic acid diet (51). Further, a growing body of evidence suggests a promising effect of oleic acid, the most predominant dietary MUFA, consumption on promoting food intake control, which may, therefore, contribute to weight management and obesity treatment (52, 53). The latter action of dietary MUFA is proposed to be triggered by oleoylethanolamide, an endogenous lipid mediator derived from MUFA, which has been recently appraised in two reviews (53, 54). These findings reinforce the notion that MUFA consumption might govern less fat accumulation and favorable fat distribution as well as endorse the recommendations of the American and Canadian Dietetic Association which allow for

consuming almost 25 % of total energy of MUFA (55). However, FA composition of North American diet does not currently contribute to obesity and metabolic abnormality risk reduction, with an excess SFA content and a MUFA level below 14% of total energy (56).

1.1.4 Dietary monounsaturated fatty acid and insulin resistance

Although the mechanisms linking dietary FA composition to IR are not completely understood yet, the preferable action of MUFA on IR might be mediated by improving cellular membrane fluidity and insulin receptor affinity (57, 58). Another suggested mechanism is modifying intramuscular fat deposition, where SFA, unlike unsaturated FAs, augment fat deposition and cause IR (59, 60). Further, the higher oxidation rate of MUFA compared to other dietary FAs may help modulate intracellular lipid balance and improve insulin sensitivity (60).

MUFA consumption has repeatedly been shown to improve insulin sensitivity measures compared to SFA. Consumption of a MUFA rich diet containing 23% of total energy for four weeks improved insulin sensitivity index values using an intravenous glucose tolerance test compared to SFA rich and carbohydrate-rich diets in subjects with abdominal obesity and type 2 diabetes (25). Three months controlled isoenergetic diet with a high MUFA content (23% of total energy) improved insulin sensitivity by 8.8% compared to a 12.5% decline following a SFA rich diet (17% of total energy) in healthy subjects (26). Further, consumption of a MUFA rich diet (>20% of total energy) for six months reduced fasting glucose, insulin, and HOMA-IR, whereas a SFA rich diet (>15% of total energy) augmented these variables in non-diabetic obese subjects (61). A systematic review of controlled feeding trials showed that replacing 5% of total energy from carbohydrate with MUFA reduced glycated haemoglobin-A1c and HOMA-IR as well as improved glucose homeostasis (62). Yet, the evidence is inconsistent (63-65), which might be attributed to the degree of obesity where the tight link between IR and obesity might hinder the

beneficial effect of MUFA on IR (65) and/or to the heterogeneity in genetic architecture which could also influence the response of IR to dietary MUFA (66).

1.1.5 Interplay of MUFA and genetic factors on obesity and IR

Numerous susceptibility genes contribute to obesity development and IR risk. Genome-wide association studies have identified 190 genetic loci associated with obesity and fat distribution (67) and 88 loci associated with type 2 diabetes risk (66). However, due to the strong link of IR with obesity and the difficulty to apply reliable insulin sensitivity measures within large cohorts, far fewer variants, to date, have been identified as associated with IR (66).

The responsiveness of individuals to dietary FA modifications was found to be modulated by genetic variants within genes that control adipogenesis and lipid turnover, appetite regulation and energy balance, as well as lipid metabolism (68-73). In contrast, little is known concerning the effect of genetic makeup in modulating the response of IR to dietary FAs. Further, dietary FA composition may also modify gene expression profiles and thus, alter obesity and IR risk (74, 75), yet, studies in humans are scarce.

Interactions between genetics and dietary factors may determine the outcome of weight-management and glycemic-control regimens as well as provoke motivation and compliance with long-term dietary changes (73, 76). For instance, the peroxisome proliferator-activated receptor gamma (*PPARγ*) rs1801282-G allele carriers had significantly less body weight and fat mass compared to CC homozygous following high-MUFA intake (69). MUFA consumption ≥13% of energy intake reduced BMI and obesity risk in the adiponectin (*ADIPOQ*) rs17300539-A allele carriers compared to the G-allele (77). Regarding insulin sensitivity and glycemic control, a MUFA rich diet was found to improve insulin sensitivity in the *ADIPOQ* rs266729-CC male homozygotes compared to SFA rich diet (78). Low adherence to Mediterranean diet, a diet rich

in MUFA, increased fasting glucose concentrations in the transcription factor 7-like 2 (*TCF7L2*) rs7903146 TT homozygotes compared to the opposing allele carriers (79). Given the aforementioned evidence, tailoring dietary intervention based on genetic architecture may help achieve better weight and glycemic control especially in genetic-predisposed subjects (80).

Nevertheless, numerous genetic variants remain to be evaluated for their effects on IR and obesity risk in response to dietary FA modification. Most of the available evidence evaluated the effect of gene-FA interaction on obesity using surrogate biomarkers, the influence of such interactions on fat mass accumulation and distinctive distribution is still missing. Furthermore, human clinical trials can provide better insights regarding the influence of genetic variants on the response of health outcomes, such as obesity and IR, to specific levels of dietary components rather than estimated dietary consumption as assessed by observational studies. Evidence from clinical trials is limited but warranted to establish personalized dietary recommendations.

1.1.6 Conclusion

MUFA consumption has been the focus of spectacular scientific interest for its promising effect on improving weight reduction and maintenance as well as enhancing insulin sensitivity and glycemic control. A genetic contribution to obesity and IR risk has been suggested, propelling research to evaluate the effect of genetic heterogeneity on the response of weight loss and insulin sensitivity to dietary MUFA. Data indicate modulatory effects of common genetic variants within genes associated with obesity and IR to the responsiveness to dietary FA modification; yet, this area of investigation is in its infancy. Hence, further investigation using human clinical trials is required to advance our knowledge of gene-MUFA interactions which may eventually lead to improving dietary recommendations that target weight and IR control.

1.2 Rationale

Growing evidence supports the role of a MUFA rich diet in reducing fat accumulation as well as ameliorating metabolic abnormalities; however, further research is warranted to assess the effect of high-MUFA consumption on fat distribution. With an increasing necessity to reduce dietary SFA, the consumption of MUFA rich oil, mainly from canola oil, is escalating in North America. In contrast, the prevalence of obesity and IR continues to rise which underscores the need for further discovery of the crossroad between diet and genetics. Therefore, investigating gene-FA interactions might illuminate the effect of inter-individual variability in genes associated with obesity and IR on the response of fat mass changes and IR measure to MUFA consumption; yet the evidence from clinical trials is missing. Further, given that health authorities in US and Canada promote 20-25% MUFA of daily total energy consumption (55), the demand is high to evaluating the mechanisms by which MUFA rich diet might induce beneficial effects on body fat deposition and distribution as well as on health outcomes including IR. Gene expression analysis can provide an insight concerning such mechanisms; nevertheless, it has been scarcely evaluated in humans. Finally, a sub-analysis was performed to evaluate the previously proposed role of high dietary MUFA on physical activity level.

Using a controlled full-feeding, double-blind, crossover, multicenter intervention trial, the chief aim of the current thesis is to delineate the impact of common genetic variants in candidate genes previously identified to be associated with obesity development on the response of body weight and fat mass changes to a high-MUFA consumption compared to a low-MUFA high-SFA diet. A secondary focus is to evaluate the influence of the genetic heterogeneity within IR-associated genes on the response of IR and glycemic control measures to dietary fatty acid

modification. The dietary intervention of this trial consisted of three phases including two high-MUFA, regular canola oil and high-oleic canola oil, and a low-MUFA high-SFA oil blend.

1.3 Objectives

This research was conducted to extend knowledge on the beneficial effects of MUFA consumption on fat mass and IR measure compared to a low-MUFA high-SFA consumption in individuals with abdominal obesity, with emphasis on identifying the influence of common genetic variants on the responsiveness of IR measure and changes in body fat mass to consumption of different levels of MUFA. Specific objectives included:

1. To investigate the efficacy of consumption of regular and high-oleic canola oils in the modulation of body composition, body fat distribution, and visceral adiposity.
2. To examine the effect of common genetic variants within obesity-associated genes on changes in body fat and distribution in response to enhanced MUFA consumption.
3. To evaluate the effect of regular and high-oleic canola oils consumption on changes in gene expression of key lipogenesis and adipogenesis-related genes in peripheral whole blood cells.
4. To assess the influence of consumption of regular and high-oleic canola oils on insulin resistance and glycemic control measures.
5. To associate common variants within candidate genes associated with IR with measures of insulin resistance in response to dietary MUFA consumption.

6. To assess spontaneous physical activity levels in response to consumption of regular and high-oleic canola oils.

1.4 Hypotheses

This research has five main hypotheses to be tested:

1. Replacing SFA with MUFA from conventional and high-oleic acid canola oils will reduce body fatness and shift body distribution toward more favorable status.
2. The consumption of high-MUFA from conventional and high-oleic acid canola oils will interact with genetic polymorphisms within genes associated with obesity to affect total and regional body fat mass.
3. The increased consumption of dietary oleic acid from conventional and high-oleic acid canola oils will change expression levels of obesity-related genes towards a less obesogenic status in peripheral whole blood cells.
4. High-MUFA content of conventional and high-oleic acid canola oils, compared to high-SFA low-MUFA oil blend, will improve insulin sensitivity and glycemic control.
5. Heterogeneity in candidate genes associated with IR will modulate the response of IR and glycemic control measures to high-MUFA consumption.
6. Substitution of SFA with MUFA in a controlled diet will increase spontaneous physical activity level.

1.5 Outline of the thesis

This thesis was written using the manuscript style and is composed of four manuscripts that follow the General Introduction (Chapter I). The first manuscript (Chapter II) summarizes

data of the literature surrounding the effects of dietary FA composition on obesity, mainly, from human studies, as well as delineates the role of the interactions between genetics and FA on obesity. Thereafter, three research manuscripts addressing each specific objective in this thesis are presented. The second manuscript in Chapter III presents a genetic basis for heterogeneity in responsiveness of fat mass loss to different levels of dietary MUFA. Chapter IV addresses an exploratory analysis of expression levels of several candidate obesity-associated genes in whole blood cells in response to high-MUFA consumption. Finally, Chapter V shows the influence of genetic heterogeneity in candidate genes associated with IR in responsiveness of IR measure to MUFA intake. Manuscript 1 has been published and Manuscript 2 and 4 are under submission. Bridges in between chapters facilitate a consistent flow of the thesis. A brief non-manuscript Chapter (Chapter VI) presents the effect of MUFA consumption on spontaneous physical activity level. The final chapter (Chapter VII) provides an overall summary of the research findings, strengths, limitations, future directions, implications, and concluding remarks.

1.6 References

1. WHO: The World Health Organization. Obesity and overweight fact sheet. Version October 2017. Internet: http://www.who.int/mediacentre/factsheets/fs311/en/index.html (accessed 13 November 2017).

2. Twells LK, Gregory DM, Reddigan J, Midodzi WK. Current and predicted prevalence of obesity in Canada: a trend analysis. CMAJ Open 2014;2(1):E18-26. doi: 10.9778/cmajo.20130016.

3. Fawcett KA, Barroso I. The genetics of obesity: FTO leads the way. Trends Genet 2010;26(6):266-74. doi: 10.1016/j.tig.2010.02.006.

4. Lyon HN, Hirschhorn JN. Genetics of common forms of obesity: a brief overview. Am J Clin Nutr 2005;82(1 Suppl):215s-7s.

5. McCarty MF. A paradox resolved: the postprandial model of insulin resistance explains why gynoid adiposity appears to be protective. Med Hypotheses 2003;61(2):173-6.

6. Min KB, Min JY. Android and gynoid fat percentages and serum lipid levels in United States adults. Clin Endocrinol (Oxf) 2015;82(3):377-87. doi: 10.1111/cen.12505.

7. Kaul S, Rothney MP, Peters DM, Wacker WK, Davis CE, Shapiro MD, Ergun DL. Dual-energy X-ray absorptiometry for quantification of visceral fat. Obesity (Silver Spring) 2012;20(6):1313-8. doi: 10.1038/oby.2011.393.

8. de Koning L, Merchant AT, Pogue J, Anand SS. Waist circumference and waist-to-hip ratio as predictors of cardiovascular events: meta-regression analysis of prospective studies. Eur Heart J 2007;28(7):850-6. doi: 10.1093/eurheartj/ehm026.

9. Lee CM, Huxley RR, Wildman RP, Woodward M. Indices of abdominal obesity are better discriminators of cardiovascular risk factors than BMI: a meta-analysis. J Clin Epidemiol 2008;61(7):646-53. doi: 10.1016/j.jclinepi.2007.08.012.

10. Wiklund P, Toss F, Weinehall L, Hallmans G, Franks PW, Nordstrom A, Nordstrom P. Abdominal and gynoid fat mass are associated with cardiovascular risk factors in men and women. J Clin Endocrinol Metab 2008;93(11):4360-6. doi: 10.1210/jc.2008-0804.

11. Ibrahim MM. Subcutaneous and visceral adipose tissue: structural and functional differences. Obes Rev 2010;11(1):11-8. doi: 10.1111/j.1467-789X.2009.00623.x.

12. Rothney MP, Catapano AL, Xia J, Wacker WK, Tidone C, Grigore L, Xia Y, Ergun DL. Abdominal visceral fat measurement using dual-energy X-ray: association with cardiometabolic risk factors. Obesity (Silver Spring) 2013;21(9):1798-802. doi: 10.1002/oby.20223.

13. Snijder MB, Visser M, Dekker JM, Seidell JC, Fuerst T, Tylavsky F, Cauley J, Lang T, Nevitt M, Harris TB. The prediction of visceral fat by dual-energy X-ray absorptiometry in the elderly: a comparison with computed tomography and anthropometry. Int J Obes Relat Metab Disord 2002;26(7):984-93. doi: 10.1038/sj.ijo.0801968.

14. Fox CS, Massaro JM, Hoffmann U, Pou KM, Maurovich-Horvat P, Liu CY, Vasan RS, Murabito JM, Meigs JB, Cupples LA, et al. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. Circulation 2007;116(1):39-48. doi: 10.1161/circulationaha.106.675355.

15. Castro AVB, Kolka CM, Kim SP, Bergman RN. Obesity, insulin resistance and comorbidities – Mechanisms of association. Arq Bras Endocrinol Metabol 2014;58(6):600-9.

16. Ross R, Freeman J, Hudson R, Janssen I. Abdominal obesity, muscle composition, and insulin resistance in premenopausal women. J Clin Endocrinol Metab 2002;87(11):5044-51. doi: 10.1210/jc.2002-020570.

17. Wajchenberg BL, Giannella-Neto D, da Silva ME, Santos RF. Depot-specific hormonal characteristics of subcutaneous and visceral adipose tissue and their relation to the metabolic syndrome. Horm Metab Res 2002;34(11-12):616-21. doi: 10.1055/s-2002-38256.

18. McLaughlin T, Lamendola C, Liu A, Abbasi F. Preferential fat deposition in subcutaneous versus visceral depots is associated with insulin sensitivity. J Clin Endocrinol Metab 2011;96(11):E1756-60. doi: 10.1210/jc.2011-0615.

19. Greenfield JR, Campbell LV. Insulin resistance and obesity. Clinics in Dermatology 2004;22(4):289-95. doi: https://doi.org/10.1016/j.clindermatol.2004.01.011.

20. Kahn BB, Flier JS. Obesity and insulin resistance. Edtion ed. J Clin Invest, 2000:473-81.

21. Patel P, Abate N. Body fat distribution and insulin resistance. Nutrients 2013;5(6):2019-27. doi: 10.3390/nu5062019.

22. Hardy OT, Czech MP, Corvera S. What causes the insulin resistance underlying obesity? Curr Opin Endocrinol Diabetes Obes 2012;19(2):81-7. doi: 10.1097/MED.0b013e3283514e13.

23. Hjorth MF, Ritz C, Blaak EE, Saris WH, Langin D, Poulsen SK, Larsen TM, Sorensen TI, Zohar Y, Astrup A. Pretreatment fasting plasma glucose and insulin modify dietary weight loss success: results from 3 randomized clinical trials. Am J Clin Nutr 2017;106(2):499-505. doi: 10.3945/ajcn.117.155200.

24. Liu X, Kris-Etherton PM, West SG, Lamarche B, Jenkins DJ, Fleming JA, McCrea CE, Pu S, Couture P, Connelly PW, et al. Effects of canola and high-oleic-acid canola oils on abdominal fat mass in individuals with central obesity. Obesity (Silver Spring) 2016;24(11):2261-8. doi: 10.1002/oby.21584.

25. Paniagua JA, Gallego de la Sacristana A, Romero I, Vidal-Puig A, Latre JM, Sanchez E, Perez-Martinez P, Lopez-Miranda J, Perez-Jimenez F. Monounsaturated fat-rich diet prevents central body fat distribution and decreases postprandial adiponectin expression induced by a carbohydrate-rich diet in insulin-resistant subjects. Diabetes Care 2007;30(7):1717-23. doi: 10.2337/dc06-2220.

26. Vessby B, Uusitupa M, Hermansen K, Riccardi G, Rivellese AA, Tapsell LC, Nalsen C, Berglund L, Louheranta A, Rasmussen BM, et al. Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. Diabetologia 2001;44(3):312-9.

27. Gillingham LG, Robinson KS, Jones PJ. Effect of high-oleic canola and flaxseed oils on energy expenditure and body composition in hypercholesterolemic subjects. Metabolism 2012;61(11):1598-605. doi: 10.1016/j.metabol.2012.04.016.

28. Krishnan S, Cooper JA. Effect of dietary fatty acid composition on substrate utilization and body weight maintenance in humans. Eur J Nutr 2014;53(3):691-710. doi: 10.1007/s00394-013-0638-z.

29. Field AE, Willett WC, Lissner L, Colditz GA. Dietary fat and weight gain among women in the Nurses' Health Study. Obesity (Silver Spring) 2007;15(4):967-76. doi: 10.1038/oby.2007.616.

30. Koh-Banerjee P, Chu NF, Spiegelman D, Rosner B, Colditz G, Willett W, Rimm E. Prospective study of the association of changes in dietary intake, physical activity, alcohol consumption, and smoking with 9-y gain in waist circumference among 16 587 US men. Am J Clin Nutr 2003;78(4):719-27.

31. Nimptsch K, Berg-Beckhoff G, Linseisen J. Effect of dietary fatty acid intake on prospective weight change in the Heidelberg cohort of the European Prospective Investigation into Cancer and Nutrition. Public Health Nutr 2010;13(10):1636-46. doi: 10.1017/s1368980009993041.

32. Simopoulos AP. An Increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. Nutrients 2016;8(3):128. doi: 10.3390/nu8030128.

33. Russo GL. Dietary n-6 and n-3 polyunsaturated fatty acids: from biochemistry to clinical implications in cardiovascular prevention. Biochem Pharmacol 2009;77(6):937-46. doi: 10.1016/j.bcp.2008.10.020.

34. Harris WS, Mozaffarian D, Rimm E, Kris-Etherton P, Rudel LL, Appel LJ, Engler MM, Engler MB, Sacks F. Omega-6 fatty acids and risk for cardiovascular disease: a science advisory from the American Heart Association Nutrition Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention. Circulation 2009;119(6):902-7. doi: 10.1161/circulationaha.108.191627.

35. Pelkman CL, Fishell VK, Maddox DH, Pearson TA, Mauger DT, Kris-Etherton PM. Effects of moderate-fat (from monounsaturated fat) and low-fat weight-loss diets on the serum lipid profile in overweight and obese men and women. Am J Clin Nutr 2004;79(2):204-12.

36. Lin L, Allemekinders H, Dansby A, Campbell L, Durance-Tod S, Berger A, Jones PJH. Evidence of health benefits of canola oil. Nutr Rev 2013;71(6):370-85. doi: 10.1111/nure.12033.

37. Piers LS, Walker KZ, Stoney RM, Soares MJ, O'Dea K. Substitution of saturated with monounsaturated fat in a 4-week diet affects body weight and composition of overweight and obese men. Br J Nutr 2003;90(3):717-27.

38. Garaulet M, Perez-Llamas F, Perez-Ayala M, Martinez P, de Medina FS, Tebar FJ, Zamora S. Site-specific differences in the fatty acid composition of abdominal adipose tissue in an obese population from a Mediterranean area: relation with dietary fatty acids, plasma lipid profile, serum insulin, and central obesity. Am J Clin Nutr 2001;74(5):585-91.

39. Sabin MA, Crowne EC, Stewart CE, Hunt LP, Turner SJ, Welsh GI, Grohmann MJ, Holly JM, Shield JP. Depot-specific effects of fatty acids on lipid accumulation in children's adipocytes. Biochem Biophys Res Commun 2007;361(2):356-61. doi: 10.1016/j.bbrc.2007.06.184.

40. Mendez MA, Popkin BM, Jakszyn P, Berenguer A, Tormo MJ, Sanchez MJ, Quiros JR, Pera G, Navarro C, Martinez C, et al. Adherence to a Mediterranean diet is associated with reduced 3-year incidence of obesity. J Nutr 2006;136(11):2934-8.

41. Alvarez-Perez J, Sanchez-Villegas A, Diaz-Benitez EM, Ruano-Rodriguez C, Corella D, Martinez-Gonzalez MA, Estruch R, Salas-Salvado J, Serra-Majem L. Influence of a Mediterranean dietary pattern on body fat distribution: results of the PREDIMED-Canarias Intervention Randomized Trial. J Am Coll Nutr 2016;35(6):568-80. doi: 10.1080/07315724.2015.1102102.

42. Di Daniele N, Petramala L, Di Renzo L, Sarlo F, Della Rocca DG, Rizzo M, Fondacaro V, Iacopino L, Pepine CJ, De Lorenzo A. Body composition changes and cardiometabolic benefits of a balanced Italian Mediterranean Diet in obese patients with metabolic syndrome. Acta Diabetol 2013;50(3):409-16. doi: 10.1007/s00592-012-0445-7.

43. Silver HJ, Kang H, Keil CD, Muldowney JA, 3rd, Kocalis H, Fazio S, Vaughan DE, Niswender KD. Consuming a balanced high fat diet for 16 weeks improves body composition, inflammation and vascular function parameters in obese premenopausal women. Metabolism 2014;63(4):562-73. doi: 10.1016/j.metabol.2014.01.004.

44. Liu X, Kris-Etherton P, West S, Lamarche B, Jenkins D, Fleming J, McCrea C, Couture P, Pu S, Jones P. Effects of canola oil and high oleic canola oil-rich diets on abdominal fat mass in individuals at risk for metabolic syndrome. New Orleans, USA: American Heart Association's Epidemiology and Prevention/Physical Activity, Nutrition and Metabolism 2013 Scientific Sessions, 2013.

45. Schmidt DE, Allred JB, Kien CL. Fractional oxidation of chylomicron-derived oleate is greater than that of palmitate in healthy adults fed frequent small meals. J Lipid Res 1999;40(12):2322-32.

46. Kien CL, Bunn JY. Gender alters the effects of palmitate and oleate on fat oxidation and energy expenditure. Obesity (Silver Spring) 2008;16(1):29-33. doi: 10.1038/oby.2007.13.

47. Alves RD, Moreira AP, Macedo VS, de Cassia Goncalves Alfenas R, Bressan J, Mattes R, Costa NM. Regular intake of high-oleic peanuts improves fat oxidation and body composition in overweight/obese men pursuing a energy-restricted diet. Obesity (Silver Spring) 2014;22(6):1422-9. doi: 10.1002/oby.20746.

48. Kien CL, Bunn JY, Ugrasbul F. Increasing dietary palmitic acid decreases fat oxidation and daily energy expenditure. Am J Clin Nutr 2005;82(2):320-6.

49. Jones PJ, Jew S, AbuMweis S. The effect of dietary oleic, linoleic, and linolenic acids on fat oxidation and energy expenditure in healthy men. Metabolism 2008;57(9):1198-203. doi: 10.1016/j.metabol.2008.04.012.

50. Soares MJ, Cummings SJ, Mamo JC, Kenrick M, Piers LS. The acute effects of olive oil v. cream on postprandial thermogenesis and substrate oxidation in postmenopausal women. Br J Nutr 2004;91(2):245-52. doi: 10.1079/bjn20031047.

51. Kien CL, Bunn JY, Tompkins CL, Dumas JA, Crain KI, Ebenstein DB, Koves TR, Muoio DM. Substituting dietary monounsaturated fat for saturated fat is associated with increased daily physical activity and resting energy expenditure and with changes in mood. Am J Clin Nutr 2013;97(4):689-97. doi: 10.3945/ajcn.112.051730.

52. Mennella I, Savarese M, Ferracane R, Sacchi R, Vitaglione P. Oleic acid content of a meal promotes oleoylethanolamide response and reduces subsequent energy intake in humans. Food Funct 2015;6(1):204-10. doi: 10.1039/c4fo00697f.

53. Bowen KJ, Kris-Etherton PM, Shearer GC, West SG, Reddivari L, Jones PJ. Oleic acid-derived oleoylethanolamide: A nutritional science perspective. Prog Lipid Res 2017. doi: 10.1016/j.plipres.2017.04.001.

54. Sihag J, Jones PJH. Oleoylethanolamide: The role of a bioactive lipid amide in modulating eating behaviour. Obes Rev 2017. doi: 10.1111/obr.12630.

55. Schwingshackl L, Hoffmann G. Monounsaturated fatty acids and risk of cardiovascular disease: synopsis of the evidence available from systematic reviews and meta-analyses. Nutrients 2012;4(12):1989-2007. doi: 10.3390/nu4121989.

56. Gillingham LG, Harris-Janz S, Jones PJ. Dietary monounsaturated fatty acids are protective against metabolic syndrome and cardiovascular disease risk factors. Lipids 2011;46(3):209-28. doi: 10.1007/s11745-010-3524-y.

57. Vessby B. Dietary fat and insulin action in humans. Br J Nutr 2000;83 Suppl 1:S91-6.

58. Lovejoy JC. The influence of dietary fat on insulin resistance. Curr Diab Rep 2002;2(5):435-40.

59. Morino K, Petersen KF, Shulman GI. Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction. Diabetes 2006;55 Suppl 2:S9-s15. doi: 10.2337/db06-S002.

60. Galgani JE, Uauy RD, Aguirre CA, Diaz EO. Effect of the dietary fat quality on insulin sensitivity. Br J Nutr 2008;100(3):471-9. doi: 10.1017/s0007114508894408.

61. Due A, Larsen TM, Hermansen K, Stender S, Holst JJ, Toubro S, Martinussen T, Astrup A. Comparison of the effects on insulin resistance and glucose tolerance of 6-mo high-monounsaturated-fat, low-fat, and control diets. Am J Clin Nutr 2008;87(4):855-62.

62. Imamura F, Micha R, Wu JH, de Oliveira Otto MC, Otite FO, Abioye AI, Mozaffarian D. Effects of saturated fat, polyunsaturated fat, monounsaturated fat, and carbohydrate on glucose-insulin homeostasis: a systematic review and meta-analysis of randomised controlled feeding trials. PLoS Med 2016;13(7):e1002087. doi: 10.1371/journal.pmed.1002087.

63. Tierney AC, McMonagle J, Shaw DI, Gulseth HL, Helal O, Saris WH, Paniagua JA, Golabek-Leszczynska I, Defoort C, Williams CM, et al. Effects of dietary fat modification on insulin sensitivity and on other risk factors of the metabolic syndrome--LIPGENE: a European

randomized dietary intervention study. Int J Obes (Lond) 2011;35(6):800-9. doi: 10.1038/ijo.2010.209.

64. Xiao C, Giacca A, Carpentier A, Lewis GF. Differential effects of monounsaturated, polyunsaturated and saturated fat ingestion on glucose-stimulated insulin secretion, sensitivity and clearance in overweight and obese, non-diabetic humans. Diabetologia 2006;49(6):1371-9. doi: 10.1007/s00125-006-0211-x.

65. Lovejoy JC, Smith SR, Champagne CM, Most MM, Lefevre M, DeLany JP, Denkins YM, Rood JC, Veldhuis J, Bray GA. Effects of diets enriched in saturated (palmitic), monounsaturated (oleic), or trans (elaidic) fatty acids on insulin sensitivity and substrate oxidation in healthy adults. Diabetes Care 2002;25(8):1283-8.

66. Brown AE, Walker M. Genetics of insulin resistance and the metabolic syndrome. Curr Cardiol Rep 2016;18(8):75. doi: 10.1007/s11886-016-0755-4.

67. Andersen MK, Sandholt CH. Recent progress in the understanding of obesity: contributions of genome-wide association studies. Curr Obes Rep 2015;4(4):401-10. doi: 10.1007/s13679-015-0173-8.

68. Garaulet M, Smith CE, Gomez-Abellan P, Ordovas-Montanes M, Lee YC, Parnell LD, Arnett DK, Ordovas JM. REV-ERB-ALPHA circadian gene variant associates with obesity in two independent populations: Mediterranean and North American. Mol Nutr Food Res 2014;58(4):821-9. doi: 10.1002/mnfr.201300361.

69. Garaulet M, Smith CE, Hernandez-Gonzalez T, Lee YC, Ordovas JM. PPARgamma Pro12Ala interacts with fat intake for obesity and weight loss in a behavioural treatment based on the Mediterranean diet. Mol Nutr Food Res 2011;55(12):1771-9. doi: 10.1002/mnfr.201100437.

70. Razquin C, Martinez JA, Martinez-Gonzalez MA, Fernandez-Crehuet J, Santos JM, Marti A. A Mediterranean diet rich in virgin olive oil may reverse the effects of the -174G/C IL6 gene variant on 3-year body weight change. Mol Nutr Food Res 2010;54 Suppl 1:S75-82. doi: 10.1002/mnfr.200900257.

71. Rosado EL, Bressan J, Hernandez JA, Martins MF, Cecon PR. [Effect of diet and PPARgamma2 and beta2-adrenergic receptor genes on energy metabolism and body composition in obese women]. Nutr Hosp 2006;21(3):317-31.

72. Corella D, Lai CQ, Demissie S, Cupples LA, Manning AK, Tucker KL, Ordovas JM. APOA5 gene variation modulates the effects of dietary fat intake on body mass index and obesity risk in the Framingham Heart Study. J Mol Med (Berl) 2007;85(2):119-28. doi: 10.1007/s00109-006-0147-0.

73. Rudkowska I, Pérusse L. Individualized weight management: what can be learned from nutrigenomics and nutrigenetics? Edtion ed. In: Bouchard C, Ordovas JM, eds. Progress in Molecular Biology and Translational Science: Academic Press, 2012:347-82.

74. Vara Prasad SS, Jeya Kumar SS, Kumar PU, Qadri SS, Vajreswari A. Dietary fatty acid composition alters 11beta-hydroxysteroid dehydrogenase type 1 gene expression in rat

retroperitoneal white adipose tissue. Lipids Health Dis 2010;9:111. doi: 10.1186/1476-511x-9-111.

75. Berná G, Oliveras-López MJ, Jurado-Ruíz E, Tejedo J, Bedoya F, Soria B, Martín F. Nutrigenetics and nutrigenomics insights into diabetes Etiopathogenesis. Edtion ed. Nutrients, 2014:5338-69.

76. Arkadianos I, Valdes AM, Marinos E, Florou A, Gill RD, Grimaldi KA. Improved weight management using genetic information to personalize a calorie controlled diet. Nutr J 2007;6:29. doi: 10.1186/1475-2891-6-29.

77. Warodomwichit D, Shen J, Arnett DK, Tsai MY, Kabagambe EK, Peacock JM, Hixson JE, Straka RJ, Province MA, An P, et al. ADIPOQ polymorphisms, monounsaturated fatty acids, and obesity risk: the GOLDN study. Obesity (Silver Spring) 2009;17(3):510-7. doi: 10.1038/oby.2008.583.

78. Perez-Martinez P, Lopez-Miranda J, Cruz-Teno C, Delgado-Lista J, Jimenez-Gomez Y, Fernandez JM, Gomez MJ, Marin C, Perez-Jimenez F, Ordovas JM. Adiponectin gene variants are associated with insulin sensitivity in response to dietary fat consumption in Caucasian men. J Nutr 2008;138(9):1609-14.

79. Corella D, Carrasco P, Sorli JV, Estruch R, Rico-Sanz J, Martinez-Gonzalez MA, Salas-Salvado J, Covas MI, Coltell O, Aros F, et al. Mediterranean diet reduces the adverse effect of the TCF7L2-rs7903146 polymorphism on cardiovascular risk factors and stroke incidence: a randomized controlled trial in a high-cardiovascular-risk population. Diabetes Care 2013;36(11):3803-11. doi: 10.2337/dc13-0955.

80. San-Cristobal R, Milagro FI, Martínez JA. Future challenges and present ethical considerations in the use of personalized nutrition based on genetic advice. Journal of the Academy of Nutrition and Dietetics 2013;113(11):1447-54. doi: https://doi.org/10.1016/j.jand.2013.05.028.

BRIDGE TO CHAPTER II

Chapter II provides an overview of the current evidence, mainly from human clinical trials, concerning the effect of dietary FA composition on obesity development. Also, this review delineates the current knowledge regarding the influence of dietary FA composition on expression levels of obesity-related genes as well as the interaction of common genetic variants within these genes with dietary FAs on obesity. The evidence reviewed in this chapter, in essence, triggered the research investigation and project presented in Chapters III, IV and VI. The following manuscript has been published in Lipids 2017; 52(10): 803–822. DOI 10.1007/s11745-017-4291-9.

CHAPTER II

MANUSCRIPT 1: LITERATURE REVIEW

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The quality of dietary fat modulates obesity and interacts with obesity-related genes

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2.1 Abstract

The prevalence of obesity is skyrocketing worldwide. The scientific evidence has associated obesity risk with many independent factors including the quality of dietary fat and genetics. Dietary fat exists as the main focus of dietary guidelines targeting obesity reduction. To prevent/minimize the adipogenic effect of dietary fatty acids (FA), intakes of long-chain saturated- and trans-FA should be reduced and substituted with unsaturated FA. The optimal proportions of dietary unsaturated FA are yet to be defined, along with a particular emphasis on the need to achieve a balanced ratio of n-3:n-6 polyunsaturated FA and to increase monounsaturated FA consumption at the expense of saturated FA. However, inter-individual variability in weight loss in response to a dietary intervention is evident, which highlights the importance of exploring gene-nutrient interactions that can further modulate the risk for obesity development. The quality of dietary fat was found to modulate obesity development by interacting with genes involved in fatty acid metabolism, adipogenesis, and the endocannabinoid system. This review summarizes the current knowledge on the effect of the quality of dietary fat on obesity phenotype and obesity-related genes. The evidence is not only supporting the modulatory effect of fat quality on obesity development but also presenting a number of interactions between obesity-related genes and the quality of dietary fat. The identified gene-FA interaction may have a clinical importance and holds a promise for the possibility of using genetically targeted dietary interventions to reduce obesity risk in the future.

2.2 Introduction

Obesity is a medical condition in which excess body fat accumulates to the extent that it may have negative impacts on health (1). Obesity is one of the largest health problems and its prevalence is skyrocketing worldwide (2-4). Many dietary, environmental, metabolic, and genetic factors are likely involved in obesity development. Dietary fat is the most energy-dense macronutrient, therefore, its consumption may influence energy balance and consequently, body weight and fatness (5, 6). Even though the quantity of dietary fat has an integral role in obesity development, the effect of the quality of dietary fat on body fat accumulation has been recognized by many researchers (6-9). Different fatty acids (FA) may vary in their obesity-inducing effect by directing the metabolism toward a pathway of either oxidation or storage (5, 10), as well as by influencing satiety and appetite sensations (11, 12). The consumption of long-chain saturated FA (SFA) and trans- FA has been linked to obesity and specifically to abdominal fat accumulation (6, 13), while unsaturated FA, polyunsaturated FA (PUFA) and monounsaturated FA (MUFA), have been found to suppress appetite as well as increase energy expenditure and fat oxidation rate, therefore, induce favorable effects on regional and total fat mass, as compared to the former two types (7-10, 12, 14).

Although excess body fat can mostly be modulated by controlling obesity-related environmental factors, genetic predisposition also plays an integral role in obesity development (15, 16). The genetic contribution to obesity explains up to 84% of body mass index (BMI) (4). Many genes have been implicated in obesity development by several genome-wide association studies (17-19). Indeed, currently, at least 190 gene loci have been found to be associated with general obesity and fat distribution (20). Furthermore, epigenetic modifications which are defined as the heritable changes in gene expression that do not involve changes to the underlying

DNA sequence, such as DNA methylation and histone modification, have been implicated in obesity development (21-23). Epigenome-wide association study identified 278 methylation sites associated with BMI (24). Although many genes are involved in the adipogenesis process, two main points remain to be elucidated; the influence of genetic variation on body weight changes and whether nutrients can modify the adipogenic effect of genes. It is therefore difficult to identify the main contributor to obesity development and fat distribution due to the complex interactions between environmental and genetic factors. However, the study of gene-diet interactions holds promise of the eventual development of a personalized prevention and treatment intervention. The study of gene-diet interactions can be divided into three main disciplines; nutrigenetics, nutrigenomics, and nutriepigenomics, which are, respectively, the science of the effect of genetic variation on dietary response, the science of the role of nutrients in gene expression via interaction with genetic structure, and the science of the role of nutrients on gene expression via epigenetic modifications (25). The type of dietary fat has been reported to influence the function/expression level of lipid metabolism and deposition-related genes, and therefore, modulate obesity risk (26, 27). A diet high in SFA mostly upregulates the lipogenic genes; SFA and trans-FA consumption increase the activity of hepatic lipogenesis and the induction of *de novo* MUFA synthesis, therefore, elevating the risk of excessive fat deposition (28, 29). In contrast, the consumption of unsaturated FA downregulates lipogenesis-related genes and upregulates FA oxidation-related genes, therefore, induces reductions in blood triglyceride and FA levels, fatty acid-cellular uptake, as well as the extent of fat deposition (28). Here, we intend to contribute to the fast-growing evidence regarding the effect of dietary fat quality on obesity. Given the role of dietary FA in obesity development and as being one important focus of nutritional intervention targeting obesity reduction, the objective is to firstly overview the

clinical trial-based evidence on the effect of dietary FA on adiposity. Secondly, an emerging evidence indicates a modulatory effect of dietary fat quality on the influence of genetic factors on fat deposition and distribution. Therefore, we identified and summarized the evidence concerning the interaction between the quality of dietary fat and adipogenesis/lipogenesis-related genes. To achieve the objective of this paper, we searched the PubMed database using different combinations of several keywords including terms "dietary fatty acids, level polyunsaturated fatty acid, level monounsaturated fatty acid, level saturated fatty acid, gene-expression, polymorphism, epigenetics, obesity, depot-specific, visceral, and subcutaneous".

2.3 Overview of the Effect of Dietary Fat on Development of Obesity

2.3.1 Fat quality and body composition

Hypocaloric diets or adjusting the amount of a specific macronutrient does not necessarily induce regional-specific fat mass reduction; however, regional fat mass shift might be induced by specific dietary component(s) (30). The quality of dietary fat has been suggested to play a vital role in fat deposition and distribution (10, 31), despite the controversy regarding the optimal type of dietary fat for obesity risk reduction. Until recently, the main dietary recommendation regarding the fat quality is to reduce the amount of SFA consumption and to increase unsaturated FA, which has been translated into higher intakes of omega-6 PUFA (n-6 PUFA), especially in a Western-type diet. In this section, we briefly review the clinical trial-based evidence.

Different dietary fats induce specific effects on body weight and fat mass (6, 7, 10). For instance, long-chain SFA consumption has been shown to enhance body adiposity and to shift fat

accumulation toward visceral depots (32, 33), where it correlates with BMI, waist-to-hip ratio, and fat mass (34-36). SFA consumption was found to have a significant direct correlation with fat cell size and number (37). A 16-week high-fat diet (50% of total fat) supplemented with nine g/day of stearate induced an increase in gynoid and total fat masses compared to energy-matched high-fat diet (38). On the other hand, medium-chain triglyceride (MCT), SFA that contains eight-12 carbon atoms, has been suggested to increase energy expenditure, thermogenesis, fat oxidation, and satiety and to reduce fat accumulation in adipocytes (39). Due to their smaller chain length, compared to long-chain FA, MCT-derived FA (MCFA) are more soluble and the majority can be absorbed rapidly with no need for esterification and incorporation into chylomicrons, therefore, swiftly metabolized for energy via β-oxidation in the liver (39, 40). These differences in the absorption and metabolism make MCFA a fast energy source that may potentially result in a negative energy balance and weight loss (39). Although the results are inconclusive regarding the effectiveness of MCT and the required dose to control body weight is not established yet, several long- and short-term studies showed an effect of MCT on increasing energy expenditure which therefore, might influence body weight and fat deposition (41-48). Evidence also indicates that MCT result in a regional fat shift in total, abdominal, and depot-specific adipose tissue as compared to long-chain FA (45). In contrast, an accumulating evidence shows negative impacts of long-chain SFA on fat deposition (8, 10, 49), nevertheless, some controversy remains over whether PUFA or MUFA induces the most favorable effect on fat mass (10, 50). Also, the optimal amount of PUFA or MUFA that can be used to replace SFA has not yet been defined (10).

There is still some discrepancy regarding the effectiveness of PUFA on weight loss and maintenance (49, 51). In the early-in-life expansion of fat depots, hyperplasia and hypertrophy

were found to be promoted by high n-6 PUFA consumption. This adipogenic effect was proven by in vitro, animal, and human studies (52). However, as compared to SFA, dietary PUFA has been connected to a smaller, less pathogenic, adipocyte cell size in human (37). Summers et al. reported a reduction in abdominal subcutaneous fat induced by the consumption of a high ratio of PUFA:SFA for five weeks, compared to a high SFA control diet, but no changes were detected in visceral adipose tissue (VAT), waist circumference (WC), or body weight (53). It is worth mentioning that in this study, the SFA diet contained a significantly higher amount of MUFA compared to the PUFA rich diet which might have influenced the effect of the control diet on body weight and blunted the possible effect of PUFA rich diets on body weight and regional fat mass. Other studies showed negative associations between total PUFA consumption and PUFA:SFA ratio with VAT and total body fat percent (35, 54). Some contradiction in the literature regarding the effect of dietary PUFA might have resulted from the differences in the proportions of n-3 and n-6 PUFA, between studies, especially given their suggested adipogenic differences. Adding omega-3 PUFA (n-3 PUFA) to a very low-calorie diet enhanced weight loss in obese women compared to very low-calorie diet (55); however, Munro and Garg have reported an absence of effect of adding n-3 PUFA on weight loss or weight maintenance to a very low-calorie diet (51). Smaller doses might be of greater benefit as reported by Crochemore et al. where they found a significant reduction in body weight and WC following 30 days supplementation of 1.5g/d of fish oil as compared to a higher dose of 2.5g/d (56). Additionally, conjugated linoleic acid has also been suggested to impose beneficial effects on body weight and body composition (57, 58). The supplementation of conjugated linoleic acid (3 g/day) and n-3 long-chain PUFA (3 g/day) for 12 weeks prevented abdominal fat deposition and increased fat-free mass and adiponectin levels, compared to a control supplementation consisting of 4.8g palm

oil and 1.2g soybean oil (58). Bjermo et al. reported that replacing SFA with n-6 PUFA promoted cardiovascular benefits but did not influence body weight (59). Diets consisting of 10% of total energy of PUFA failed to provide any additional favorable effect on whole body weight, abdominal fat mass, or VAT mass as compared to a macronutrient-matched low-PUFA (5% of total energy) diet over a 12-week period; however, both diets induced significant reductions in body weight and fat mass (60, 61). It is worth mentioning that the low-PUFA diet in this study had a higher MUFA content (15% of total energy compared to 10% in the PUFA enriched diet), therefore, the higher MUFA content might induce favorable effects on obesity indices and blunted the effect of the high PUFA diet.

MUFA consumption has been gaining a particular recent scientific interest regarding its beneficial effects on disease and obesity risks (49, 62-65). Adherence to a diet rich in MUFA was not associated with obesity over the long term (66, 67). Even though Garaulet et al. have found no association between MUFA consumption and fat cell size; however, the MUFA content of adipocytes has been found to be inversely related to fat cell number, which might indicate a preferential effect of MUFA in reducing hyperplasia (37). Supplementing a high-fat diet with oleic acid (nine g/day) for 16 weeks induced a reduction in android fat mass as compared to a placebo high-fat diet (50% of total fat) (38). No additional beneficial effects on body weight, WC, or total fat mass were detected following hypocaloric MUFA rich diet (22.5% MUFA, 11.25% PUFA, and 11.25% SFA) as compared to the hypocaloric diet with equal FA proportions (10% MUFA, 10% PUFA, and 10% SFA) in obese non-diabetic and type 2 diabetic individuals. However, this lack of effect might have been caused by diabetes-related changes in metabolism and/or lack of statistical power, as the authors indicated (68). Nimptsch et al. found an inverse association between MUFA intake and prospective weight gain, while saturated and PUFA

intakes were linearly associated with prospective weight gain (49). Additionally, a MUFA-rich diet containing 23% of total energy was found to prevent further fat accumulation in abdominal region in abdominally obese subjects (30). A randomized, cross-over, ad libitum feeding study found that a MUFA-rich diet (~ 23% MUFA 11% SFA of total energy) induced small but significant reductions in body weight and fat mass, as compared to a high SFA diet (~ 24% SFA and ~ 13% MUFA of total energy) (8). The reductions in fat mass and body weight following the MUFA enriched diet were observed despite the lack of significant differences in the intake of energy or fat during the two diets (8). Although Gillingham et al. reported lack of statistical differences in body weight and total and regional fat mass following diets rich in either SFA, high-oleic canola oil, or flaxseed-high-oleic canola oil blend, this study found a trend toward significance (p=0.055) in the ratio of android:gynoid fat mass (5). The observed lower ratio of android:gynoid fat mass (mean ± SEM, 1.11±0.04) following the MUFA diet as compared to a diet rich in either SFA (1.12±0.04) or PUFA (1.13±0.04) might indicate a favorable effect of MUFA on body fat redistribution. Another trial was conducted to examine the effects of consumption of different dietary oils varying in their unsaturated fat contents on body composition and fat distribution in a randomized controlled crossover weight-maintenance full-feeding design (69). This study has revealed favorable effects of MUFA-rich diets, including regular canola oil or high oleic canola oil, in terms of reducing body weight and android fat mass as compared to a diet rich in flax/safflower oil blend (PUFA-rich diet) (7). Additionally, there is general agreement regarding the effectiveness of the Mediterranean diet, rich in α-linolenic acid and oleic acid, for weight and fat mass reduction/maintenance (64, 67, 70-74). In summary, there is general agreement that substituting long-chain SFA with unsaturated FA induces beneficial

effects on weight loss. While the evidence regarding the effect of PUFA consumption on obesity is showing less clear conclusion, MUFA holds promise for effective dietary substitution for SFA.

2.3.2 Fat quality and energy expenditure

Levels of energy expenditure and fat oxidation are important regulators of fat deposition. A decrease in the level of fat oxidation, especially in skeletal muscle; the main site for fat oxidation, can dramatically increase obesity risk (75, 76). Fat oxidation rate is greatly influenced by insulin sensitivity which in turn might be affected by dietary fat quality. An animal trial showed that consumption of n-6 PUFA appeared to prevent insulin resistance compared to a diet rich in SFA (77). In n-3 PUFA-fed rats, compared to lard (SFA)-fed rats PUFAs increased lipid oxidation, enhanced the activation of AMP-activated protein kinase (AMPK; a key enzyme in cellular energy homeostasis), and improved insulin signaling in skeletal muscle (78). Furthermore, oleic acid may contribute to the prevention of palmitate-induced insulin resistance by ameliorating palmitate-induced mitochondrial dysfunction in rat (79). As compared to SFA consumption, consumption of MUFA (80, 81) and supplementation of long-chain n-3 PUFA (82, 83) were reported to improve insulin sensitivity in humans. The beneficial effects of unsaturated FA on insulin sensitivity and energy metabolism might be translated into a reduction in obesity risk.

Most of the existing evidence supports higher rates of whole body FA oxidation and diet-induced thermogenesis following unsaturated FA consumption compared to SFA consumption; however, data regarding the oxidative differences between PUFA and MUFA are less clear (10). The lower oxidation rates of saturated compared to unsaturated FA reduce the energy expenditure level and increase their propensity to be stored in adipocytes (10, 84-86). The data regarding PUFA effects on energy expenditure are less conclusive, especially because most

studies that evaluated the effect of PUFA on energy expenditure have not specified the source of this fat, i.e. n-3 versus n-6, which would decrease the applicability of their results. An increasing body of evidence indicates increasing increments in fat oxidation rate, diet-induced thermogenesis, and energy expenditure with higher dietary MUFA levels (10, 14, 86-88). In a recent randomized parallel-arm clinical trial, increased fat oxidation and reduced body fatness were observed in men who received 56 g/day of conventional or high-oleic peanuts for four weeks during an energy-restricted diet, as compared to a hypocaloric-control diet (9). Even though Gillingham et al. have found that replacing SFA with high-oleic canola oil or flaxseed-high-oleic canola oil blend did not induce favorable effects on energy expenditure or fat oxidation, they reported a trend (p=0.055) for increasing the ratio of android/gynoid fat mass following a high-oleic canola diet compared to a diet rich in flaxseed-high-oleic canola (5). Piers et al. have reported a small but significant reduction in body weight following a four-week of MUFA-rich diet compared to SFA-rich diet; however, no significant differences in energy expenditure or substrate oxidation rate were found between the two diets (8). Furthermore, Kien and Bunn reported increases in FA oxidation in women and an elevation of energy expenditure in men following 28 days of high oleic acid-rich diet, as compared to a diet rich in palmitic acid (14). Substituting dietary palmitic acid with oleic acid was associated with increased physical activity and resting energy expenditure (88).

Moreover, in normal weight subjects, the majority of studies reported no short-term difference in fat oxidation following a high-fat meal enriched with MUFA, PUFA, or SFA (10, 87, 89), while high PUFA and MUFA meals elevated the thermic effect of a meal compared to SFA (90-93). The contradiction on the effect of different dietary fats on rates of fat oxidation and energy expenditure might be due to the variations in study design or methodology between

different trials. However, Krishnan and Cooper, have comprehensively reviewed the available evidence from the long-term intervention and postprandial meal challenge regarding the effect of different dietary FA in a high-fat diet/meal on substrate utilization, diet-induced thermogenesis, and fat oxidation. These authors report that unsaturated FA, especially MUFA, greatly increase diet induced thermogenesis and fat oxidation as compared to SFA (MUFA ≥ PUFA > SFA) (10). The question remains whether or not the increased fat oxidation rate seen with MUFA consumption is sufficient to induce fat mass loss.

2.3.3 Fat quality and energy consumption

The quality of dietary fat has been suggested to modulate energy consumption, the second arm of the energy balance equation, therefore, modulate obesity risk. Energy intake might be reduced when the ghrelin levels are reduced and peptide-YY, cholecystokinin, and glucagon-like peptide-1 levels are increased (11, 94). A high-fat meal that is enriched with either PUFA or MUFA was found to suppress postprandial ghrelin levels in obese women to a significantly greater extent compared to a high-fat high-SFA meal, while a PUFA rich meal was able to significantly elevate peptide-YY compared to SFA and MUFA-rich meals in women with obesity (11). However, these changes in satiety signals were not translated into shifts in the subjective appetite ratings (VAS) or changes in subsequent energy intake (11). Also, Strik et al. did not support a specific role of SFA, MUFA, and PUFA on the subjective rating of hunger and fullness sensations as well as the subsequent food consumption over six-h period (95). Another study has reported an increase in cholecystokinin levels, a significant increase in fullness, and a reduction in hunger following PUFA and MUFA liquid meals in healthy subjects as compared to SFA meal (94). Current evidence supports the effect of the degree of FA saturation on satiety subjective and physiological appetite responses (11, 90, 94); however, it has yet to be elucidated

whether MUFA or PUFA is most influential in controlling energy consumption (94, 96). Anandamide, a FA neurotransmitter derived from the eicosatetraenoic acid and an essential n-6 polyunsaturated fatty acid, acts as an endogenous ligand for the cannabinoid receptor that stimulates appetite sensation which might increase energy consumption (12, 97). Adequate consumption of long-chain n-3 PUFA and obtaining an n-6:n-3 PUFA ratio as close as possible to unity can blunt any possible adverse effects of arachidonic acid metabolites and hyperactivity of the cannabinoid system (98, 99). Furthermore, the high oleic acid content of the diet was suggested to control appetite sensation and therefore, reduce energy intake by elevating post-prandial oleoylethanolamide level, which itself regulates food intake by influencing metabolic and reward systems (12, 97, 100). This finding is novel in humans and might hold promise for using oils rich in oleic acid to control energy intake (12). Taking the above-mentioned evidence, different dietary FA differently impact body composition, energy expenditure, and energy consumption, therefore, influence obesity risk in unalike ways. In summary, the optimal diet for weight reduction/maintenance has to be balanced in n-6:n-3 ratio, as close as possible to unity, and enriched in MUFA at the expense of long-chain SFA.

Nutrition is one of the most important factors that modulate the influence of genes on obesity risk. The aim of studying gene-nutrient interactions is to ultimately use genetic profile to personalize dietary recommendation. Given the effect of dietary fat quality on obesity, the next section summarizes the evidence concerning the effect of fat quality on lipogenesis/adipogenesis-related genes.

2.4 The Effect of Dietary Fat on Adipogenesis-Related Genes

As noted earlier, gene-nutrient interactions may eventually help to prevent/control obesity in high-risk individuals by designing personalized nutritional strategies. Dietary FA have been found to modulate the expression levels of several genes and transcriptional factors that are involved in cellular responsiveness to metabolic signals and/or the regulation of lipid and energy metabolism (28, 30). The effects of dietary FA on obesity might depend on their chain length and desaturation degree. The two transcription factors, sterol regulatory element binding protein-1 (*SREBP1*) and peroxisome proliferator activated receptor (*PPAR*), have emerged as key mediators of gene regulation by dietary FA, therefore, the literature has mainly focused on the interaction of FA with these two transcriptional factors. Three main mechanisms underlie the gene-FA interaction; dietary influences on membrane FA composition, transcriptional regulation, and post-transcriptional processes (101). Epigenetics (non-DNA sequence-related heritable changes) and genetics (DNA sequence-related heritable changes) may interact to modify the expression of genes, thereby, the risk for obesity and associated disease.

2.4.1 Obesity-related transcriptional factors

Of the obesity-related genes that have been identified, two main transcriptional factors, *PPAR* and *SREBP1* exist as key mediators of the effect of hormones and nutrients on the expression of fat accumulation-related genes (102). **Figure 2.1** displays the effects of these two transcriptional factors on adipogenesis and lipogenesis processes in adipose tissue and liver.

PPARs are transcriptional factors expressed mainly in skeletal muscle, liver, and brown adipose tissue which regulate the expression levels of several genes. PPARs are involved in the regulation of cellular differentiation, development, and the metabolism of lipid, protein, and carbohydrate (28, 103). Three types of PPARs have been identified; beta (PPARδ), alpha

(PPARα), and gamma (PPARγ) (103, 104). Little is known about the role of PPARδ in lipid metabolism; however, the activation of adipocyte PPARδ might be involved in obesity risk reduction by enhancing the expression of FA oxidation- and utilization-related genes (105). The activation of PPARα enhances FA oxidation pathways, increases energy expenditure, reduces *de novo* synthesis of FA in the liver and adipose tissue, and promotes thermogenesis in brown adipose tissue, therefore, reducing the level of fat deposition (28, 106-108). PPARα activity is downregulated by insulin signals and enhanced by high free FA levels. *PPARγ*, expressed mainly in white and brown adipose tissue, is involved in adipocyte differentiation, and regulates the metabolic and endocrine functions of adipose tissue (28, 109). In contrast to PPARα, PPARγ activation promotes the expression levels of lipogenesis- and adipogenesis-related genes yet ameliorate whole body insulin resistance and hypertriglyceridemia (28, 109-112). PPARγ is part of the adipocyte differentiation program and its activity is induced by higher levels of insulin, SREBP1, and FA (113). PPARγ has been identified as a regulator of the levels of leptin, resistin, and adiponectin, modulating adipokines levels reflects an important role of PPARγ as a determinant of dietary intake, energy homeostasis, and obesity risk (104, 112). The effect of PPARγ on adiposity levels has been verified by the strong positive relationships between the expression levels of *PPARγ* and several lipogenic enzymes as well as VAT adiponectin levels (31). Therefore, PPARs influence obesity risk differently; the activation of PPARδ and PPARα reduces obesity risk by increasing energy expenditure and fat oxidation, in contrast, PPARγ induces lipogenesis and adipogenesis and augments obesity risk.

SREBPs are membrane-bound transcriptional factors that are involved in the uptake and biosynthesis of FA and triglyceride (SREBP1) as well as cholesterol (SREBP2) (114). Insulin is an important regulator of SREBP transcriptional and protein levels, also insulin-induced

activation of several lipogenesis genes requires upregulation of the expression levels of *SREBP1* (28, 115). Therefore, the induction of SREBP1 and PPARγ activities increases the lipogenesis and adipogenesis of triglyceride in adipose tissue by augmenting the expression of several adipogenic and lipogenic enzymes (28, 31).

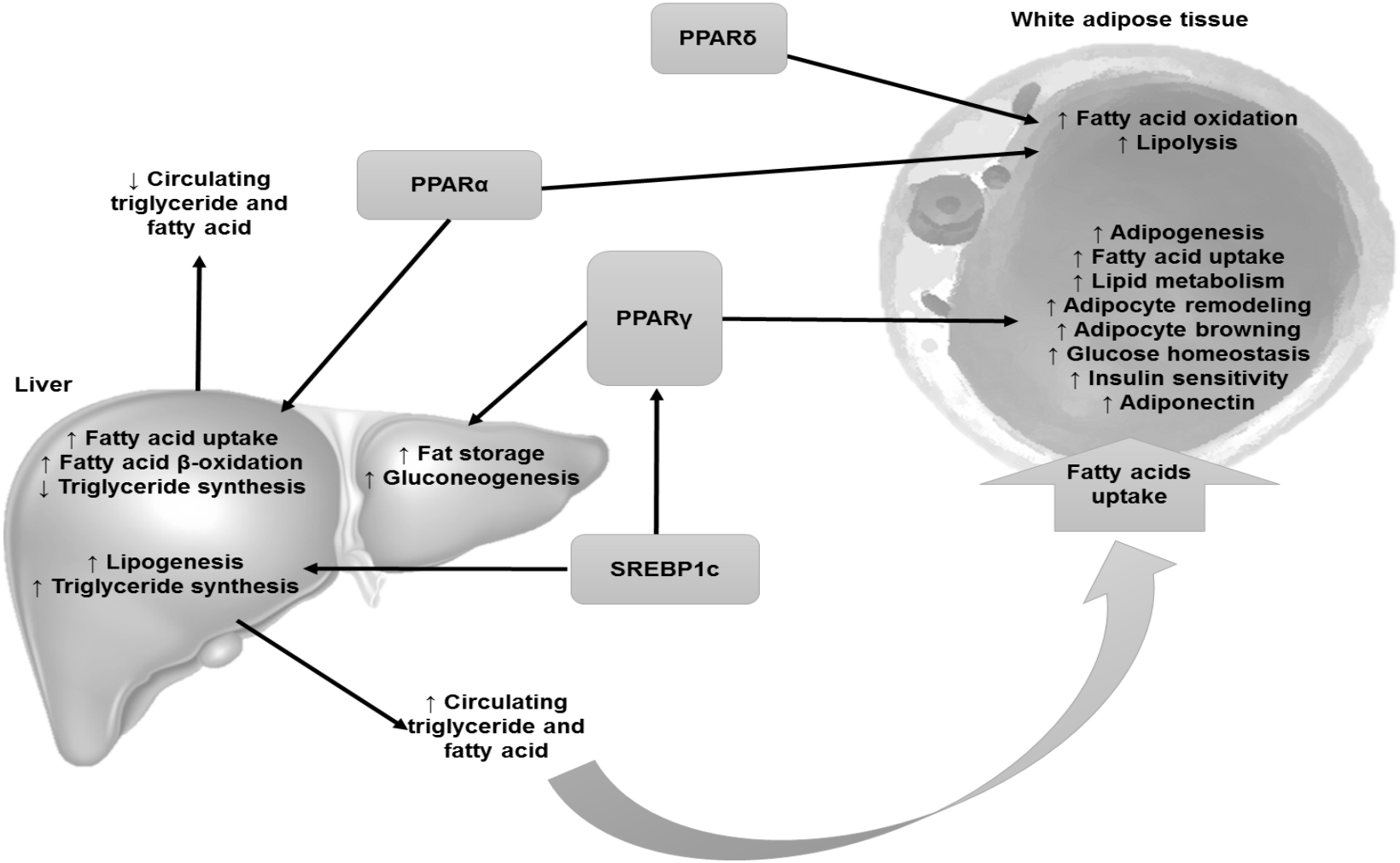

Figure 2.1: The effect of peroxisome proliferator-activated receptor alpha (*PPARα*), gamma (*PPARγ*), and delta (*PPARδ*) and sterol regulatory element-binding proteins-1c (*SREBP1c*) on adipogenesis and lipogenesis processes in adipose tissue and liver

2.4.2 The role of saturated fatty acid on the expression of obesity-related genes

The effects of SFA on the expression of adipogenesis and lipogenesis-related genes were found to be modulated by the FA chain length. Short-chain FA consumption reduced the *PPAR*-induced activation of some hepatic lipogenic genes which might reduce the obesity risk (28). Adipose tissue-MCFA have shown an ability to downregulate the expression levels of *PPARγ* and its downstream metabolic target genes as well as to improve insulin sensitivity and reduce lipoprotein lipase activity in adipocyte (40). On the other hand, long-chain SFA consumption has been reported to upregulate the activity of SREBP1c and PPARγ which activates the lipogenic genes including stearoyl-CoA desaturase, fatty acid synthase, and acetyl-CoA carboxylase (26, 29). Furthermore, the expression levels of *PPARγ*- and *PPARγ*-induced activation of adipogenic and lipogenic genes have been enhanced by a diet rich in trans-FA (28). SFA is suggested to modulate gene expression by changing the cellular membrane composition which will change the intra-cellular substrate-to-product ratio of a target gene and therefore, will enhance/prevent its expression levels. For instance, SFA can mediate its effect on SREBP1 by changing the cellular membrane composition to possess more SFA which would decrease cholesterol and SFA levels in the endoplasmic reticulum, and therefore, increases SREBP1 maturation (28). An animal trial presented a suggested mechanism by which the quality of dietary fat might modulate abdominal obesity; compared to SFA, the expression levels of 11β-hydroxysteroid dehydrogenase type 1 (*11β-HSD1*) and CCAAT-enhancer binding protein-α (*C/EBP-α*) in retroperitoneal fat depot were higher following trans-FA and lower following PUFA consumption (116). 11β-HSD-1 converts inactive corticosteroids into potent glucocorticoids, expressed in various tissues including liver and adipose tissue. Its expression is regulated by *C/EBP-α*, and the higher activity of this enzyme is associated with an increased

VAT mass (109, 116). The amplification of glucocorticoids action by SFA and trans-FA as compared to PUFA may increase fat deposition and insulin resistance and may, partly, explain the adipogenic and pathogenic effect of SFA and trans-FA (116).

2.4.3 The role of unsaturated fatty acid on the expression of obesity-related genes

Unsaturated FA suppress expression levels of FA biosynthesis-related genes and upregulate FA oxidation-related gene expression levels. PUFA consumption, in general, has been found to induce activation of PPARα and several genes related to lipid oxidation and thermogenesis, resulting in an increased FA oxidation rate and a reduced risk of adipogenesis (28, 117, 118). PUFA consumption has been found to suppress expression levels of many lipogenic genes and chiefly reduce the expression level of *SREBP1* by either inhibiting mRNA gene expression or obstructing the proteolytic processing of SREBP1 precursor (26, 113, 117, 119). PUFA consumption induces an opposite effect of SFA consumption on *SREBP1* expression by the same mechanism reported earlier (28). These two types of dietary PUFA induce different effects on fat metabolism and deposition; n-6 PUFA is suggested to promote fat deposition, while n-3 PUFA might induce the reverse effect. N-3 PUFA consumption was found to be associated with a reduction in *PPARγ* expression level and has been reported to suppress *de novo* lipogenesis (106, 120). In contrast, n-6 PUFA have been reported to induce early activation of PPARγ and are recognized for having higher PPARγ-affinity as compared to other FA (121). *SREBP1c* expression level was reported to be related directly to adipose tissue content of n-6 PUFA, while n-3 (α-linoleic acid) PUFA was found to blunt the lipogenic effects of n-6 PUFA by reducing expression levels of *SREBP1c* and fatty acid synthase in adipose tissue in rats (31). Dietary n-3 PUFA was found to inhibit expression and nuclear transcription of *SREBP1* therefore, reducing lipogenesis (106). Such possible adipogenic effects of n-6 PUFA are

suggested to be manifested when the ratio of n-6:n-3 PUFA is larger than unity, which is clearly demonstrated in a typical Western diet (31, 122). A high dose of n-3 PUFA is required to blunt the adipogenic effect of n-6 PUFA (31, 123), the optimal n-6:n-3 PUFA ratio can be best achieved by supplementing n-3 PUFA and reducing dietary n-6 PUFA consumption in a diet. The favorable effects of n-3 PUFA are suggested to be induced by changing the n-6:n-3 PUFA ratio of cellular membrane and plasma phospholipid contents which might alter the enzymatic activity and metabolic pathways, as well as by reducing the cellularity of adipose tissue and inhibiting hyperplasia and hypertrophy of adipocytes (123, 124). Additionally, the beneficial effects of a balanced n-6:n-3 PUFA ratio might also be explained by an anti-inflammatory effect of n-3 PUFA which would ameliorate inflammatory status and enhance insulin sensitivity, and therefore, modulate adipogenesis and lipogenesis (31, 99). Downregulation of the expression level of adiponectin gene was found to be induced by dietary enrichment with soybean high in n-6 PUFA, coconut high in MCFA, and lard high in LC-SFA, as compared to fish oil (125), which might partly emphasize the superiority of n-3 PUFA in improving insulin sensitivity. Improving insulin sensitivity can modulate energy and lipid metabolism.

Little has been reported on the effect of MUFA on expression levels of adipogenic/lipogenic genes. Dietary MUFA upregulate the expression level of mitochondrial uncoupling protein-2 gene as well as *PPARγ* and its target genes which might alter energy balance, insulin resistivity, and adiposity level (118, 126). However, MUFA consumption failed to suppress lipogenesis or to modulate PPARα activity as compared to a high n-3 PUFA diet in rats (106). On the other hand, MUFA consumption in an isocaloric diet was found to prevent the reduction in postprandial adiponectin gene expression levels in peripheral tissue, improve insulin resistance, and induce preferential body fat distribution i.e., lower central deposition, compared

to high carbohydrate diet in insulin resistance subjects (30). Given the above-mentioned evidence from the clinical trials regarding the beneficial effects of dietary MUFA on obesity, further research on the effect of MUFA consumption on the expression levels of adipogenesis/lipogenesis-related genes is warranted to understand the underlying mechanism behind the favorable effects of MUFA.

2.4.4 Fat quality and epigenetic modifications of obesity-related genes

Evidence regarding the role of dietary FA on epigenetic regulations of the expression levels of obesity-related genes is scarce (127). However, a few studies have demonstrated the epigenetic effects of FA consumption, especially PUFA, on obesity control and prevention mostly in animal models and cultured cells (22, 127-129). The methylation of several obesity-related genes was found to be influenced by the proportions of PUFA:SFA, MUFA:SFA, and unsaturated fatty acid:SFA, indicating a role of epigenetics in the physiological responses to the quality of dietary fat (130). Palmitate treatment in human islets demonstrated changes in DNA methylation and gene expression of 67 BMI-related genes (131). Additionally, in cultured cells, arachidonic acid, palmitic acid, and oleic acid altered the DNA methylation of several genes that regulate pathological processes including obesity; arachidonic acid and palmitic acid induced similar DNA methylation profile compared to oleic acid-induced profile (132). In a seven-week parallel controlled clinical trial in 31 healthy normal-weight adults (133), overfeeding of SFA changed the methylation in the promoter regions of 125 genes and significantly altered the expression of 28 genes including acyl-CoA oxidase-1 in subcutaneous adipose tissue. In the same trial, PUFA altered the methylation in the promoter regions of 1797 genes including fat mass and obesity- associated gene (FTO) but did not significantly affect gene expression levels. The epigenetic targets, DNA methylation and histone modifications, were found to be involved

in n-3 PUFA-induced modification of leptin expression in the adipose tissue of diet-induced obese mice (134). Nutritional epigenomics is an emerging field of research and since dietary fat quality is a main contributor to obesity development, rigorous clinical trials are needed to directly assess the effect of FA on epigenetic modifications of obesity-related genes.

2.4.5 The interaction between fat quality and single nucleotide polymorphisms of obesity-related genes

Finally, dietary FA have been found to modulate the association between body composition and many genetic variants of obesity-related genes (111, 135-141), **Table 2.1** summarizes available evidence on dietary fatty acid-adipogenic gene interactions. The data show promising evidence of interactions between dietary FA and several polymorphisms located in genes that can co-ordinate the adipogenesis process either via a metabolically-evident pathway such as PPARγ or via a suggested mechanism such as Circadian Locomotor Output Cycles Kaput. However, many factors might modulate the interactions between dietary FA and genes, including ethnicity and many environmental factors, therefore, large-scale ethnic-specific longitudinal studies that account for many environmental, behavioral, and cultural variables are still required in order to eliminate as much as possible of the confounders that might affect gene-nutrient interactions. The long-term aim for studying the gene-nutrient interaction is to instruct genotype-specific personalized dietary recommendations for the prevention and treatment of obesity. To achieve this goal, more randomized well-controlled clinical trials are needed to validate gene-nutrient interactions.

Table 2.1: The effect of dietary fatty acid-gene interaction on body composition

Reference	Participant/Diet	Gene/ SNP	Response
(140)	1465 overweight or obese men and women in Spanish/ behavioural treatment program	*PPARγ*[a]/ rs1801282	Subjects carrying minor allele G were significantly less obese than the homozygous major subjects CC when the MUFA intake was high
(112)	2141 USA women/ survey	*PPARγ*[a]/ rs1801282	• MUFA consumption was inversely associated with BMI in the minor allele carriers • PUFA:SFA ratio was directly associated with lower BMI among the homozygous major allele carrier
(158)	720 French-Canadian descent men and women/ survey	*PPARγ*[a]/ rs1801282	An increase in WC was associated with an increased level of saturated fat intake in homozygous major allele carrier but not among carriers of minor allele
(101)	308 European normal and obese, men and women/ prospective survey	*PPARγ*[a]/ rs1801282	Carriers of the minor allele G who consumed high amounts of arachidonic acid had a significantly higher risk of obesity than the carriers of major allele
(159)	60 Brazilian obese women/ a randomized trial	*PPARγ*[a]/ rs1801282	• The habitual MUFA intake inversely correlated with fat oxidation and BMI in the obese G carriers • A lower PUFA intake in the long-term trial was associated with an increase in the respiratory quotient only in G carriers
(111)	592 Caucasian nondiabetic men and women/ survey	*PPARγ*[a]/ rs1801282	An inverse association was detected between PUFA:SFA ratio and BMI among G carriers
(160)	3356 mixed population men and women/ survey	*PPARγ*[a]/ rs1801282	Carriers of G allele who consumed high PUFA:SFA ratio had the greatest reduction in visceral fat

(Continued)

Table 2.1: Continued

Reference	Participant/Diet	Gene/ SNP	Response
(101)	308 European normal and obese, men and women/ prospective survey	*PPARγ*[a] / rs3856806	Carriers of the T allele who consumed low amounts of linoleic acid had a significantly higher risk of obesity than the carriers of the major allele
(161)	60 Spanish obese women/ controlled trial	*PPARγ*[a] / rs1801282 *ADRB2*[b]/ rs1042714	• Carriers of a combination of major rs1801282 CC and minor rs1042714 GG who consumed a high MUFA diet had an increase in carbohydrate oxidation and a smaller weight loss • A combination of heterozygous of both SNPs had a greater energy expenditure and basal and postprandial fat oxidation after a short-term high SFA diet
(162)	260 Spanish obese Men and women/ a randomized trial	*ADRB3*[c]/ rs4994	No significant differences on weight, BMI, WC, or fat mass in either genotype group with both diets
(135)	2163 European ancestry and Puerto Rican obese men and women/ survey	*FTO*[d]/ rs9939609 and rs1121980	Carriers of rs9939609 AA allele or rs1121980 TT allele had a higher BMI than the other genotypes only when they had a high SFA intake
(163)	776 Spanish women and men/ a randomized trial	*FTO*[d]/ rs9939609	No interaction between the nutritional intervention and the polymorphism was found
(164)	233 Spanish obese subjects men and women/ a randomized trial	*FTO*[d]/ rs9939609	Lower levels of BMI, weight and fat mass were detected after a PUFA diet in A allele carriers than TT genotype subjects
(101)	308 European normal-weight and obese, men and women/ prospective survey	*LEP*[e]/ rs7799039	Carriers of A allele who consumed a higher amount of linoleic acid were found to be at a lower risk for obesity
(165)	200 Mexican normal-weight and obese men and women/ survey	*LEPR*[f]/ rs8179183 and rs1137101	Carriers of rs1137101 G allele with a high SFA intake had ~3 times higher risk of obesity

(Continued)

Table 2.1: Continued

Reference	Participant/Diet	Gene/ SNP	Response
(166)	1083 Caucasian origin men and women/ survey	*ADIPOQ*[g]/ rs17300539	A allele carriers who consumed a higher amount of MUFA had a lower BMI and a reduced obesity risk
(101)	308 European normal and obese, men and women/ prospective survey	*HSL*[h]/ rs34845087	Carriers of the G allele who consumed high amounts of arachidonic acid showed a tendency to a lower risk of obesity than the carriers of the major allele
(138)	1171 African American and European American men and women/ survey	*LPL*[i]/ rs320	A higher intake of PUFA was associated with lower BMI and WC in major TT allele carriers
(167)	391 Spanish obese men and women/ a randomized trial	*GLP1R*[j]/ rs6923761	Lack of association of rs6923761 with weight loss after either high MUFA or PUFA hypocaloric diets was reported
(168)	1100 European descent men and women/ prospective survey	*CLOCK*[k]/ rs1801260	Minor allele GG carriers who consumed a high SFA intake had larger WC than non-carriers
(102)	11091 European men and women/ survey	*CEBPB*[L]/ rs4253449	Higher intake of MUFA, PUFA, and SFA were associated with a higher risk of weight gain among minor allele carriers
(169)	258 Spanish obese men and women/ a randomized trial	*CNR1*[m]/ rs1049353	No significant differences on weight, BMI, WC, or fat mass in either genotype group with both diets
(170)	1147 Puerto Ricans men and women/ survey	*BDNF*[n]/ rs6265	• Compared to A allele carriers, a low PUFA intake in men with the GG was associated with a higher BMI, and higher waist and hip circumferences • Compared to A allele carriers, a lower BMI was associated with a high PUFA intake in women with GG • Compared to A allele carriers, a lower BMI and hip circumference were associated with a high SFA intake in women with the GG

(Continued)

Table 2.1: Continued

Reference	Participant/Diet	Gene/ SNP	Response
(171)	2212 white women and men/ analysis of two independent study	*REV-ERB-ALPHA* circadian[o]/ rs2314339	Consuming a higher amount of MUFA in the Mediterranean population was associated with a higher BMI in major GG allele carriers
(172)	1754 French men and women/ survey	*STAT3*[p]/ rs8069645, rs744166, rs2306580, rs2293152, rs10530050	A high SFA consumption in individuals carrying ≥2 risk alleles was associated with an increased risk of abdominal obesity compared with those carrying ≤1 risk alleles
(173)	268 Normal-weight and obese, black and white women in South African	*IL-6*[q]/ rs1800795, rs2069845, rs1554606	In white women only, a higher n-3 PUFA intake and a reduced n-6:n-3 PUFA ratio were associated with a lower BMI in carriers of rs1800795 C allele, rs1554606 T allele and rs2069845 AG genotype
(174)	737 Spanish men and women/ a randomized trial	*IL-6*[q]/ rs1800795	CC carriers had the lowest body weight gain following the Mediterranean diet supplemented with virgin olive oil (but not with nuts)
(175)	378 normal-weight and obese, black and white women in South African	*TNFα*[R]/ rs361525	• An increased SFA intake in A carriers was associated with a higher adiposity increase as compared to GG • An increased MUFA intake in A carriers was associated with a higher weight as compared to GG
(176)	223 Black South African obese and normal weight women/ survey	*TNFα*[R]/ rs1800629	No gene-nutrient interaction was found in any dietary fatty acid with this SNP on obesity
(101)	308 European normal and obese, men and women/ prospective survey	*TNFα*[R]/ rs1800629	Interactions of high intakes of linoleic acid and arachidonic acid and A allele carriers showed a higher obesity risk
(177)	261 Spanish obese men and women/ survey	*TNFα*[R]/ rs1800629	No significant differences on weight, BMI, WC, or fat mass in either genotype group with both diets

(Continued)

Table 2.1: Continued

Reference	Participant/Diet	Gene/ SNP	Response
(178)	936 European origin men and women/ survey	*TACE*^{S}/ rs10495563	The deleterious association of the A allele with obesity was observed in subjects with a low n-6 PUFA intake
(165)	200 Mexican normal-weight and obese men and women/ survey	*APOA5*^{I}/ rs662799 and rs3135506	Carriers of rs3135506 GG genotype with a high SFA consumption had an increased risk of obesity
(179)	2280 non-Hispanic whites men and women/ survey	*APOA5*^{I}/ rs662799 and rs3135506	Rs662799 C minor allele carriers with the high MUFA intake had a lower obesity risk compared with TT carriers
(180)	3462 Whites and Hispanic men and women/ follow-up survey	*APOA2*^{V}/ rs5082	CC genotype with a high SFA intake was associated with a higher obesity prevalence
(181)	2071 Puerto Ricans origin and Northern European ancestry men and women/ survey	*APOA2*^{V}/ rs5082	CC carriers who consumed a greater amount of SFA from a high-fat dairy foods had a greater BMI than those who consume less dairy fat
(182)	737 Iranian diabetic patients men and women/ survey	*APOA2*^{V}/ rs5082	Carriers of CC allele with a high SFA intake had higher BMI
(165)	200 Mexican normal-weight and obese men and women/ survey	*APOA2*^{V}/ rs3813627 and rs5082	No interaction was found between SFA intake and APOA2 SNPs
(183)	1225 Spanish overweight and obese men and women/ survey	*APOA2*^{V}/ rs5082	A high SFA intake was associated with a greater WC in minor allele homozygotes CC compared with non-minor allele carriers
(184)	4602 Asian and Mediterranean obese men and women/ survey	*APOA2*^{V}/ rs5082	In both populations, the CC genotype was associated with a greater degree of obesity in those consuming a high SFA diet
(185)	700 Iranian diabetic men and women/cross-sectional	*APOB*^{W}/ Ins/Del SNP within the first exon	A high n-3 PUFA consumption decreased the obesity risk in carriers of the Del allele of APOB gene

(Continued)

Table 2.1: Continued

Reference	Participant/Diet	Gene/ SNP	Response
(186)	920 Puerto Rican origin men and women/ survey	*LRP1*[z]/ rs1799986, rs715948, and rs1800191	A high intake of SFA was associated with a higher BMI, and higher waist and hip circumferences in carriers of rs1799986 T allele compared to CC. High palmitic and stearic acids induced the strongest effect on BMI
(187)	Meta-analyses using data from 14 studies of US and European whites and 4 of African Americans	*LRP1*[z]/ 7 SNPs in White and 12 SNPs in African Americans	A high intake of SFA was associated with an increased BMI, and higher waist and hip circumferences among whites, but not African Americans, TT allele carriers of rs2306692

ADIPOQ, Adiponectin; *ADRB2*, adrenergic receptor beta2; *ADRB3*, adrenergic beta-3- receptor; *APOA2*, apolipoprotein-A2; *APOA5*, apolipoprotein-A5; *APOB*, apolipoprotein-B; *BDNF*, brain-derived neurotrophic factor; BMI, body mass index; *CEBPB*, CCAAT/enhancer binding protein-b; *CLOCK*, circadian locomotor output cycles kaput; *CNR1*, cannabinoid receptor-1; *GLP1R*, glucagon-like peptide-1 receptor; *HSL*, hormone sensitive lipase; *IL-6*, interleukin-6; *LEP*, leptine; *LEPR*, leptin receptor; *LPL*, lipoprotein lipase; *LRP1*, low density lipoprotein related receptor protein-1; MUFA, monounsaturated fatty acid; *PPARγ*, peroxisome proliferator-activated receptors gamma; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; SNP, single nucleotide polymorphism; *STAT3*, signal transducer and activator of transcription-3; *TACE*, tumor necrosis factor alpha converting enzyme; *TNFα*, tumor necrosis factor alpha; WC: waist circumference.

Role in obesity: [a]PPARγ role in obesity is fully described in the text; [b]ADRB2 is involved in energy expenditure and lipolysis; [c]ADRB3 is located mainly in adipose tissue and is involved in the regulation of lipolysis and thermogenesis; [d]*FTO* is considered as the most significant candidate gene that implicated in the development of obesity; however, the function of this gene is unknown yet; [e]LEP is involved in energy balance by inhibiting hunger; [f]LEPR mutation of the *LEPR* gene results in leptin insensitivity, hyperphagia, morbid obesity; [g]ADIPOQ is involved in insulin sensitivity and energy metabolism; [h]HSL plays a crucial role in the hydrolysis of triacylglycerol; [i]LPL hydrolases triglyceride which are carried on lipoprotein; [j]GLP1R is involved in satiety control; [k]CLOCK is involved in metabolic alterations; [L]CEBPB activation induces the division of cells; [m]CNR1 is an endogenous ligand of endocannabinoid system and is located in several brain areas and adipose tissue; [n]BDNF suppresses food intake and is involved in the conversion of white fat into brown fat in adipose tissue; [o]REV-ERB-ALPHA circadian functions as a coordinator of metabolic responses that adhere to circadian patterns; [p]STAT3 is a transcription factor and it is involved in body weight control, glucose homeostasis, leptin sensitivity, and appetite control; [q]IL-6 higher circulating concentrations of this cytokine have been associated with obesity and visceral adipose tissue deposition; [R]TNFα polymorphism was associated with increased risk for obesity; [S]TACE releases the soluble form of tumor necrosis factor from their membrane-bound precursors, TNF-α is overexpressed and highly released from the adipose tissue of obese humans; [t]APOA5 is regarded as an important modulator in the metabolism of triglycerides; [v]APOA2 is involved in lipid metabolism and in the regulation of food intake; [w]ApoB is the key protein involved in the synthesis and secretion of chylomicrons and very-low-density lipoprotein; [z]LRP1 mediates lipoprotein remnant uptake, is highly expressed in adipocyte, and has been suggested to play a role in adipogenesis, cell signaling, and energy and glucose metabolism.

2.5 Abdominal Fat Depots Vary in their Adipogenic Effect

Quantifying of total and regional fat mass as well as differentiating between the types of fat depots provide a clear picture regarding the pathogenesis of obesity and therefore, better insight to disease risk (142). Subcutaneous adipose tissue (SCAT) is the adipocyte that lies just beneath the skin, while adipocyte that accumulates around the vital organ in the abdominal cavity is known as VAT. Studies have shown strong and independent associations between VAT depot, rather than SCAT, with metabolic abnormalities and chronic disease risks (143-147). Anatomical, cellular, molecular, physiological and clinical differences exist between VAT and SCAT (143, 148). These variations between SCAT and VAT might influence their responsiveness to nutritional intervention. For instance, a systematic review showed a dissimilar responsiveness of VAT and SCAT to weight loss energy-restricted diet (149).

Depot-specific variations were also detected in mRNA expression levels and in the activity of several fat metabolism-related genes and transcriptional factors. Compared to VAT, higher activation levels of *PPARγ* and several downstream genes have been detected in SCAT (110, 150), in contrast, the expression of *PPARα* in VAT has been reported to be higher than SCAT (151). These two properties might reflect biological defense mechanism against visceral obesity by directing fat deposition from VAT to SCAT. On the other hand, *PPARγ* expression was found to be elevated in VAT of obese subjects as compared to non-obese subjects, which indicating obesity-induced metabolic changes that might be involved in directing fat deposition toward the pathological fat depot (152). Guiu-Jurado et al. have reported a significant inverse association between BMI and two lipogenic genes (fatty acid synthase and acetyl-CoA carboxylase-1) as well as with *PPARα* in SCAT, while expression levels of these genes remained similar in VAT regardless of the degree of obesity (153). Furthermore, the level of hormone-

sensitive lipase in SCAT was found to be 2 fold lower than corresponding levels in VAT, explaining the elevated level of lipolysis in VAT of obese women (154). The expression level of apolipoprotein-E, which plays an important role in lipid metabolism and modulate adipocyte substrate metabolism and storage, was found to be lower in VAT as compared to SCAT (155). Also, a depot-specific difference in perilipin levels was suggested to contribute to differences in basal lipolysis and adipocyte size; levels of perilipin were found to be higher in VAT as compared to SCAT in women (154). Additionally, nesfatin-1, an anorexigenic peptide, has been found to be secreted in a depot-specific manner, preferentially by SCAT (156). No data are available regarding the effect of adipocyte-produced nesfatin-1 on energy homeostasis yet; however, this might reflect a favorable role of SCAT over VAT. Therefore, the detected depot-specific variation in expression levels of adipokines as well as adipogenic/lipogenic genes might contribute to the variation in the adipogenic and pathogenic capacity of VAT.

Despite the well-established variations in the health risk posed by VAT versus SCAT, little is known about the effects of different dietary treatments on each depot. However, compared to SFA and MUFA dietary mixture, dietary docosahexaenoic acid has significantly reduced mRNA fatty acid synthase, hormone sensitive lipase, lipoprotein lipase, and leptin in VAT in rats; however, the expression levels of these enzymes in SCAT were not affected by n-3 PUFA (157). A direct association was found between n-6 PUFA concentrations and *SREBP1c* expression levels in both fat depots; however, the responsiveness degree was higher in VAT than SCAT (31).

Additionally, the FA composition of adipocytes is suggested to reflect FA composition over a long period (150). SCAT and VAT have shown variations in their FA contents which may indicate variations in the pathological capacities of different FA. The degree of obesity, central

obesity, and visceral fat were negatively associated with MUFA and n-3 PUFA content in visceral region (32). Also, MUFA were found to contribute to the highest proportion of SCAT region as compared to its proportion in VAT (32, 150). On the other hand, a positive association was found between n-6 PUFA content in adipose tissue and central obesity, and no difference in PUFA content has been found between SCAT and VAT (32). Sabin et al. reported that palmitic acid induced significantly higher lipid accumulation in VAT as compared to oleate, in contrast, the latter was associated with fat deposition in SCAT (33). Dietary PUFA perfectly mirrored their content in adipose tissue, particularly SCAT (1, 150, 157); however, MUFA and SFA contents in adipocyte might not mirror their abundance in the diet because of the endogenous synthesis of these FA (1, 150). In this regard, the SFA content in adipocyte imposes some controversy; Garaulet et al. reported a depot-specific variation in SFA contents of obese adult subjects, where SFA was significantly higher in the perivisceral region (32). In contrast, Caron-Jobin et al. presented a negative correlation between BMI in obese women and the adipocyte stearic acid content, which is the most abundant SFA in Western diet and can be also synthesized endogenously (150). This latter finding can be explained by an increased level of *de novo* synthesis of unsaturated FA from dietary SFA, especially given the finding that the increased desaturation level in SCAT is associated with overall obesity and with higher levels of stearoyl-CoA desaturase mRNA expression and protein abundance (150). Therefore, the quality of dietary fat can even modulate the distribution of body fat and further affect obesity level and downstream health consequences.

2.6 Conclusion

Evidence suggests that the quality of fat determines the risk of fat deposition and fat distribution. This effect is directed, partly, by modulating the expression levels of adipogenic and lipogenic genes, via genetic and epigenetic modifications, as well as by the interaction with many genetic variants. However, more clinical trials are needed to validate the interactions between FA and adipogenesis-related genes. The current clinical trial-based evidence supports the beneficial effects of the increased consumption of dietary unsaturated fatty acids, especially MUFA, at the expense of SFA on weight loss/maintenance. The identified interactions between obesity-related genes and the quality of dietary fat may eventually lead to the use of genetic profile for tailoring nutritional intervention provided to individuals or population sub-group.

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2.7 References

1. Garaulet M, Hernandez-Morante JJ, Tebar FJ, Zamora S. Relation between degree of obesity and site-specific adipose tissue fatty acid composition in a Mediterranean population. Nutrition 2011;27(2):170-6. doi: 10.1016/j.nut.2010.01.004.

2. Bastien M, Poirier P, Lemieux I, Despres JP. Overview of epidemiology and contribution of obesity to cardiovascular disease. Prog Cardiovasc Dis 2014;56(4):369-81. doi: 10.1016/j.pcad.2013.10.016.

3. James WP. The epidemiology of obesity: the size of the problem. J Intern Med 2008;263(4):336-52. doi: 10.1111/j.1365-2796.2008.01922.x.

4. Fall T, Ingelsson E. Genome-wide association studies of obesity and metabolic syndrome. Mol Cell Endocrinol 2014;382(1):740-57. doi: 10.1016/j.mce.2012.08.018.

5. Gillingham LG, Robinson KS, Jones PJ. Effect of high-oleic canola and flaxseed oils on energy expenditure and body composition in hypercholesterolemic subjects. Metabolism 2012;61(11):1598-605. doi: 10.1016/j.metabol.2012.04.016.

6. Field AE, Willett WC, Lissner L, Colditz GA. Dietary fat and weight gain among women in the Nurses' Health Study. Obesity (Silver Spring) 2007;15(4):967-76. doi: 10.1038/oby.2007.616.

7. Liu X, Kris-Etherton PM, West SG, Lamarche B, Jenkins DJ, Fleming JA, McCrea CE, Pu S, Couture P, Connelly PW, et al. Effects of canola and high-oleic-acid canola oils on abdominal fat mass in individuals with central obesity. Obesity (Silver Spring) 2016;24(11):2261-8. doi: 10.1002/oby.21584.

8. Piers LS, Walker KZ, Stoney RM, Soares MJ, O'Dea K. Substitution of saturated with monounsaturated fat in a 4-week diet affects body weight and composition of overweight and obese men. Br J Nutr 2003;90(3):717-27.

9. Alves RD, Moreira AP, Macedo VS, de Cassia Goncalves Alfenas R, Bressan J, Mattes R, Costa NM. Regular intake of high-oleic peanuts improves fat oxidation and body composition in overweight/obese men pursuing a energy-restricted diet. Obesity (Silver Spring) 2014;22(6):1422-9. doi: 10.1002/oby.20746.

10. Krishnan S, Cooper JA. Effect of dietary fatty acid composition on substrate utilization and body weight maintenance in humans. Eur J Nutr 2014;53(3):691-710. doi: 10.1007/s00394-013-0638-z.

11. Stevenson JL, Clevenger HC, Cooper JA. Hunger and satiety responses to high-fat meals of varying fatty acid composition in women with obesity. Obesity (Silver Spring) 2015;23(10):1980-6. doi: 10.1002/oby.21202.

12. Mennella I, Savarese M, Ferracane R, Sacchi R, Vitaglione P. Oleic acid content of a meal promotes oleoylethanolamide response and reduces subsequent energy intake in humans. Food Funct 2015;6(1):204-10. doi: 10.1039/c4fo00697f.

13. Koh-Banerjee P, Chu NF, Spiegelman D, Rosner B, Colditz G, Willett W, Rimm E. Prospective study of the association of changes in dietary intake, physical activity, alcohol consumption, and smoking with 9-y gain in waist circumference among 16 587 US men. Am J Clin Nutr 2003;78(4):719-27.

14. Kien CL, Bunn JY. Gender alters the effects of palmitate and oleate on fat oxidation and energy expenditure. Obesity (Silver Spring) 2008;16(1):29-33. doi: 10.1038/oby.2007.13.

15. Lyon HN, Hirschhorn JN. Genetics of common forms of obesity: a brief overview. Am J Clin Nutr 2005;82(1 Suppl):215s-7s.

16. Emdin CA, Khera AV, Natarajan P, Klarin D, Zekavat SM, Hsiao AJ, Kathiresan S. Genetic association of waist-to-hip ratio with cardiometabolic traits, type 2 diabetes, and coronary heart disease. Jama 2017;317(6):626-34. doi: 10.1001/jama.2016.21042.

17. Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, Powell C, Vedantam S, Buchkovich ML, Yang J, et al. Genetic studies of body mass index yield new insights for obesity biology. Nature 2015;518(7538):197-206. doi: 10.1038/nature14177.

18. Fox CS, Liu Y, White CC, Feitosa M, Smith AV, Heard-Costa N, Lohman K, Johnson AD, Foster MC, Greenawalt DM, et al. Genome-wide association for abdominal subcutaneous and visceral adipose reveals a novel locus for visceral fat in women. PLoS Genet 2012;8(5):e1002695. doi: 10.1371/journal.pgen.1002695.

19. Wang T, Ma X, Peng D, Zhang R, Sun X, Chen M, Yan J, Wang S, Yan D, He Z, et al. Effects of obesity related genetic variations on visceral and subcutaneous fat distribution in a Chinese population. Sci Rep 2016;6:20691. doi: 10.1038/srep20691.

20. Andersen MK, Sandholt CH. Recent progress in the understanding of obesity: contributions of genome-wide association studies. Curr Obes Rep 2015;4(4):401-10. doi: 10.1007/s13679-015-0173-8.

21. Aslibekyan S, Demerath EW, Mendelson M, Zhi D, Guan W, Liang L, Sha J, Pankow JS, Liu C, Irvin MR, et al. Epigenome-wide study identifies novel methylation loci associated with body mass index and waist circumference. Obesity (Silver Spring) 2015;23(7):1493-501. doi: 10.1002/oby.21111.

22. Milagro FI, Mansego ML, De Miguel C, Martinez JA. Dietary factors, epigenetic modifications and obesity outcomes: progresses and perspectives. Mol Aspects Med 2013;34(4):782-812. doi: 10.1016/j.mam.2012.06.010.

23. Wang B, Gao W, Li J, Yu C, Cao W, Lv J, Pang Z, Cong L, Wang H, Wu X, et al. Methylation loci associated with body mass index, waist circumference, and waist-to-hip ratio in Chinese adults: an epigenome-wide analysis. Lancet 2016;388 Suppl 1:S21. doi: 10.1016/s0140-6736(16)31948-1.

24. Wahl S, Drong A, Lehne B, Loh M, Scott WR, Kunze S, Tsai PC, Ried JS, Zhang W, Yang Y, et al. Epigenome-wide association study of body mass index, and the adverse outcomes of adiposity. Nature 2017;541(7635):81-6. doi: 10.1038/nature20784.

25. Gaboon NEA. Nutritional genomics and personalized diet. Egyptian Journal of Medical Human Genetics 2011;12(1):1-7. doi: http://dx.doi.org/10.1016/j.ejmhg.2011.02.001.

26. Flowers MT, Ntambi JM. Role of stearoyl-coenzyme A desaturase in regulating lipid metabolism. Curr Opin Lipidol 2008;19(3):248-56. doi: 10.1097/MOL.0b013e3282f9b54d.

27. Popeijus HE, Saris WH, Mensink RP. Role of stearoyl-CoA desaturases in obesity and the metabolic syndrome. Int J Obes (Lond) 2008;32(7):1076-82. doi: 10.1038/ijo.2008.55.

28. Reynes B, Palou M, Palou A. Gene expression modulation of lipid and central energetic metabolism related genes by high-fat diet intake in the main homeostatic tissues. Food Funct 2017;8(2):629-50. doi: 10.1039/c6fo01473a.

29. Sampath H, Miyazaki M, Dobrzyn A, Ntambi JM. Stearoyl-CoA desaturase-1 mediates the pro-lipogenic effects of dietary saturated fat. J Biol Chem 2007;282(4):2483-93. doi: 10.1074/jbc.M610158200.

30. Paniagua JA, Gallego de la Sacristana A, Romero I, Vidal-Puig A, Latre JM, Sanchez E, Perez-Martinez P, Lopez-Miranda J, Perez-Jimenez F. Monounsaturated fat-rich diet prevents central body fat distribution and decreases postprandial adiponectin expression induced by a carbohydrate-rich diet in insulin-resistant subjects. Diabetes Care 2007;30(7):1717-23. doi: 10.2337/dc06-2220.

31. Muhlhausler BS, Cook-Johnson R, James M, Miljkovic D, Duthoit E, Gibson R. Opposing effects of omega-3 and omega-6 long chain polyunsaturated Fatty acids on the expression of lipogenic genes in omental and retroperitoneal adipose depots in the rat. J Nutr Metab 2010;2010. doi: 10.1155/2010/927836.

32. Garaulet M, Perez-Llamas F, Perez-Ayala M, Martinez P, de Medina FS, Tebar FJ, Zamora S. Site-specific differences in the fatty acid composition of abdominal adipose tissue in an obese population from a Mediterranean area: relation with dietary fatty acids, plasma lipid profile, serum insulin, and central obesity. Am J Clin Nutr 2001;74(5):585-91.

33. Sabin MA, Crowne EC, Stewart CE, Hunt LP, Turner SJ, Welsh GI, Grohmann MJ, Holly JM, Shield JP. Depot-specific effects of fatty acids on lipid accumulation in children's adipocytes. Biochem Biophys Res Commun 2007;361(2):356-61. doi: 10.1016/j.bbrc.2007.06.184.

34. Ward KD, Sparrow D, Vokonas PS, Willett WC, Landsberg L, Weiss ST. The relationships of abdominal obesity, hyperinsulinemia and saturated fat intake to serum lipid levels: the Normative Aging Study. Int J Obes Relat Metab Disord 1994;18(3):137-44.

35. Larson DE, Hunter GR, Williams MJ, Kekes-Szabo T, Nyikos I, Goran MI. Dietary fat in relation to body fat and intraabdominal adipose tissue: a cross-sectional analysis. Am J Clin Nutr 1996;64(5):677-84.

36. Hariri N, Gougeon R, Thibault L. A highly saturated fat-rich diet is more obesogenic than diets with lower saturated fat content. Nutr Res 2010;30(9):632-43. doi: 10.1016/j.nutres.2010.09.003.

37. Garaulet M, Hernandez-Morante JJ, Lujan J, Tebar FJ, Zamora S. Relationship between fat cell size and number and fatty acid composition in adipose tissue from different fat depots in overweight/obese humans. Int J Obes (Lond) 2006;30(6):899-905. doi: 10.1038/sj.ijo.0803219.

38. Silver HJ, Kang H, Keil CD, Muldowney JA, 3rd, Kocalis H, Fazio S, Vaughan DE, Niswender KD. Consuming a balanced high fat diet for 16 weeks improves body composition, inflammation and vascular function parameters in obese premenopausal women. Metabolism 2014;63(4):562-73. doi: 10.1016/j.metabol.2014.01.004.

39. Mumme K, Stonehouse W. Effects of medium-chain triglycerides on weight loss and body composition: a meta-analysis of randomized controlled trials. J Acad Nutr Diet 2015;115(2):249-63. doi: 10.1016/j.jand.2014.10.022.

40. Han J, Hamilton JA, Kirkland JL, Corkey BE, Guo W. Medium-chain oil reduces fat mass and down-regulates expression of adipogenic genes in rats. Obes Res 2003;11(6):734-44. doi: 10.1038/oby.2003.103.

41. Tsuji H, Kasai M, Takeuchi H, Nakamura M, Okazaki M, Kondo K. Dietary medium-chain triacylglycerols suppress accumulation of body fat in a double-blind, controlled trial in healthy men and women. J Nutr 2001;131(11):2853-9.

42. Kasai M, Nosaka N, Maki H, Negishi S, Aoyama T, Nakamura M, Suzuki Y, Tsuji H, Uto H, Okazaki M, et al. Effect of dietary medium- and long-chain triacylglycerols (MLCT) on accumulation of body fat in healthy humans. Asia Pac J Clin Nutr 2003;12(2):151-60.

43. Clegg ME. Medium-chain triglycerides are advantageous in promoting weight loss although not beneficial to exercise performance. Int J Food Sci Nutr 2010;61(7):653-79. doi: 10.3109/09637481003702114.

44. Rego Costa AC, Rosado EL, Soares-Mota M. Influence of the dietary intake of medium chain triglycerides on body composition, energy expenditure and satiety: a systematic review. Nutr Hosp 2012;27(1):103-8. doi: 10.1590/s0212-16112012000100011.

45. Roynette CE, Rudkowska I, Nakhasi DK, Jones PJ. Structured medium and long chain triglycerides show short-term increases in fat oxidation, but no changes in adiposity in men. Nutr Metab Cardiovasc Dis 2008;18(4):298-305. doi: 10.1016/j.numecd.2006.11.004.

46. St-Onge MP, Bosarge A. Weight-loss diet that includes consumption of medium-chain triacylglycerol oil leads to a greater rate of weight and fat mass loss than does olive oil. Am J Clin Nutr 2008;87(3):621-6.

47. St-Onge MP, Bourque C, Jones PJ, Ross R, Parsons WE. Medium- versus long-chain triglycerides for 27 days increases fat oxidation and energy expenditure without resulting in changes in body composition in overweight women. Int J Obes Relat Metab Disord 2003;27(1):95-102. doi: 10.1038/sj.ijo.0802169.

48. St-Onge MP, Jones PJ. Greater rise in fat oxidation with medium-chain triglyceride consumption relative to long-chain triglyceride is associated with lower initial body weight and greater loss of subcutaneous adipose tissue. Int J Obes Relat Metab Disord 2003;27(12):1565-71. doi: 10.1038/sj.ijo.0802467.

49. Nimptsch K, Berg-Beckhoff G, Linseisen J. Effect of dietary fatty acid intake on prospective weight change in the Heidelberg cohort of the European Prospective Investigation into Cancer and Nutrition. Public Health Nutr 2010;13(10):1636-46. doi: 10.1017/s1368980009993041.

50. Moussavi N, Gavino V, Receveur O. Could the quality of dietary fat, and not just its quantity, be related to risk of obesity? Obesity (Silver Spring) 2008;16(1):7-15. doi: 10.1038/oby.2007.14.

51. Munro IA, Garg ML. Dietary supplementation with n-3 PUFA does not promote weight loss when combined with a very-low-energy diet. Br J Nutr 2012;108(8):1466-74. doi: 10.1017/s0007114511006817.

52. Muhlhausler BS, Ailhaud GP. Omega-6 polyunsaturated fatty acids and the early origins of obesity. Curr Opin Endocrinol Diabetes Obes 2013;20(1):56-61. doi: 10.1097/MED.0b013e32835c1ba7.

53. Summers LK, Fielding BA, Bradshaw HA, Ilic V, Beysen C, Clark ML, Moore NR, Frayn KN. Substituting dietary saturated fat with polyunsaturated fat changes abdominal fat distribution and improves insulin sensitivity. Diabetologia 2002;45(3):369-77. doi: 10.1007/s00125-001-0768-3.

54. Cardel M, Lemas DJ, Jackson KH, Friedman JE, Fernandez JR. Higher intake of PUFAs is associated with lower total and visceral adiposity and higher lean mass in a racially diverse sample of children. J Nutr 2015;145(9):2146-52. doi: 10.3945/jn.115.212365.

55. Kunesova M, Braunerova R, Hlavaty P, Tvrzicka E, Stankova B, Skrha J, Hilgertova J, Hill M, Kopecky J, Wagenknecht M, et al. The influence of n-3 polyunsaturated fatty acids and very low calorie diet during a short-term weight reducing regimen on weight loss and serum fatty acid composition in severely obese women. Physiol Res 2006;55(1):63-72.

56. Crochemore IC, Souza AF, de Souza AC, Rosado EL. omega-3 polyunsaturated fatty acid supplementation does not influence body composition, insulin resistance, and lipemia in women with type 2 diabetes and obesity. Nutr Clin Pract 2012;27(4):553-60. doi: 10.1177/0884533612444535.

57. Blankson H, Stakkestad JA, Fagertun H, Thom E, Wadstein J, Gudmundsen O. Conjugated linoleic acid reduces body fat mass in overweight and obese humans. J Nutr 2000;130(12):2943-8.

58. Sneddon AA, Tsofliou F, Fyfe CL, Matheson I, Jackson DM, Horgan G, Winzell MS, Wahle KW, Ahren B, Williams LM. Effect of a conjugated linoleic acid and omega-3 fatty acid mixture on body composition and adiponectin. Obesity (Silver Spring) 2008;16(5):1019-24. doi: 10.1038/oby.2008.41.

59. Bjermo H, Iggman D, Kullberg J, Dahlman I, Johansson L, Persson L, Berglund J, Pulkki K, Basu S, Uusitupa M, et al. Effects of n-6 PUFAs compared with SFAs on liver fat, lipoproteins, and inflammation in abdominal obesity: a randomized controlled trial. Am J Clin Nutr 2012;95(5):1003-12. doi: 10.3945/ajcn.111.030114.

60. Tapsell L, Batterham M, Huang XF, Tan SY, Teuss G, Charlton K, Oshea J, Warensjo E. Short term effects of energy restriction and dietary fat sub-type on weight loss and disease risk factors. Nutr Metab Cardiovasc Dis 2010;20(5):317-25. doi: 10.1016/j.numecd.2009.04.007.

61. Tan SY, Batterham M, Tapsell L. Increased intake of dietary polyunsaturated fat does not promote whole body or preferential abdominal fat mass loss in overweight adults. Obes Facts 2011;4(5):352-7. doi: 10.1159/000333433.

62. Venturini D, Simao AN, Urbano MR, Dichi I. Effects of extra virgin olive oil and fish oil on lipid profile and oxidative stress in patients with metabolic syndrome. Nutrition 2015;31(6):834-40. doi: 10.1016/j.nut.2014.12.016.

63. Potenza MV, Mechanick JI. The metabolic syndrome: definition, global impact, and pathophysiology. Nutr Clin Pract 2009;24(5):560-77. doi: 10.1177/0884533609342436.

64. Bergouignan A, Momken I, Schoeller DA, Simon C, Blanc S. Metabolic fate of saturated and monounsaturated dietary fats: the Mediterranean diet revisited from epidemiological evidence to cellular mechanisms. Prog Lipid Res 2009;48(3-4):128-47. doi: 10.1016/j.plipres.2009.02.004.

65. Pelkman CL, Fishell VK, Maddox DH, Pearson TA, Mauger DT, Kris-Etherton PM. Effects of moderate-fat (from monounsaturated fat) and low-fat weight-loss diets on the serum lipid profile in overweight and obese men and women. Am J Clin Nutr 2004;79(2):204-12.

66. Bes-Rastrollo M, Sabate J, Gomez-Gracia E, Alonso A, Martinez JA, Martinez-Gonzalez MA. Nut consumption and weight gain in a Mediterranean cohort: The SUN study. Obesity (Silver Spring) 2007;15(1):107-16. doi: 10.1038/oby.2007.507.

67. Mendez MA, Popkin BM, Jakszyn P, Berenguer A, Tormo MJ, Sanchez MJ, Quiros JR, Pera G, Navarro C, Martinez C, et al. Adherence to a Mediterranean diet is associated with reduced 3-year incidence of obesity. J Nutr 2006;136(11):2934-8.

68. Brunerova L, Smejkalova V, Potockova J, Andel M. A comparison of the influence of a high-fat diet enriched in monounsaturated fatty acids and conventional diet on weight loss and metabolic parameters in obese non-diabetic and Type 2 diabetic patients. Diabet Med 2007;24(5):533-40. doi: 10.1111/j.1464-5491.2007.02104.x.

69. Senanayake VK, Pu S, Jenkins DA, Lamarche B, Kris-Etherton PM, West SG, Fleming JA, Liu X, McCrea CE, Jones PJ. Plasma fatty acid changes following consumption of dietary oils containing n-3, n-6, and n-9 fatty acids at different proportions: preliminary findings of the Canola Oil Multicenter Intervention Trial (COMIT). Trials 2014;15:136. doi: 10.1186/1745-6215-15-136.

70. Austel A, Ranke C, Wagner N, Gorge J, Ellrott T. Weight loss with a modified Mediterranean-type diet using fat modification: a randomized controlled trial. Eur J Clin Nutr 2015;69(8):878-84. doi: 10.1038/ejcn.2015.11.

71. Alvarez-Perez J, Sanchez-Villegas A, Diaz-Benitez EM, Ruano-Rodriguez C, Corella D, Martinez-Gonzalez MA, Estruch R, Salas-Salvado J, Serra-Majem L. Influence of a Mediterranean dietary pattern on body fat distribution: results of the PREDIMED-Canarias Intervention Randomized Trial. J Am Coll Nutr 2016;35(6):568-80. doi: 10.1080/07315724.2015.1102102.

72. Yannakoulia M, Aggelopoulou D, Skenderi K, Koinaki S, Yiannakouris N. A Mediterranean-like breakfast affects energy intake and appetite-related feelings. Int J Food Sci Nutr 2014;65(7):899-902. doi: 10.3109/09637486.2014.931359.

73. Di Daniele N, Petramala L, Di Renzo L, Sarlo F, Della Rocca DG, Rizzo M, Fondacaro V, Iacopino L, Pepine CJ, De Lorenzo A. Body composition changes and cardiometabolic benefits of a balanced Italian Mediterranean Diet in obese patients with metabolic syndrome. Acta Diabetol 2013;50(3):409-16. doi: 10.1007/s00592-012-0445-7.

74. Razquin C, Martinez JA, Martinez-Gonzalez MA, Mitjavila MT, Estruch R, Marti A. A 3 years follow-up of a Mediterranean diet rich in virgin olive oil is associated with high plasma antioxidant capacity and reduced body weight gain. Eur J Clin Nutr 2009;63(12):1387-93. doi: 10.1038/ejcn.2009.106.

75. Berggren JR, Boyle KE, Chapman WH, Houmard JA. Skeletal muscle lipid oxidation and obesity: influence of weight loss and exercise. Am J Physiol Endocrinol Metab 2008;294(4):E726-32. doi: 10.1152/ajpendo.00354.2007.

76. Kim JY, Hickner RC, Cortright RL, Dohm GL, Houmard JA. Lipid oxidation is reduced in obese human skeletal muscle. Am J Physiol Endocrinol Metab 2000;279(5):E1039-44.

77. Lee JS, Pinnamaneni SK, Eo SJ, Cho IH, Pyo JH, Kim CK, Sinclair AJ, Febbraio MA, Watt MJ. Saturated, but not n-6 polyunsaturated, fatty acids induce insulin resistance: role of intramuscular accumulation of lipid metabolites. J Appl Physiol (1985) 2006;100(5):1467-74. doi: 10.1152/japplphysiol.01438.2005.

78. Cavaliere G, Trinchese G, Bergamo P, De Filippo C, Mattace Raso G, Gifuni G, Putti R, Moni BH, Canani RB, Meli R, et al. Polyunsaturated fatty acids attenuate diet induced obesity and insulin resistance, modulating mitochondrial respiratory uncoupling in rat skeletal muscle. PLoS One 2016;11(2):e0149033. doi: 10.1371/journal.pone.0149033.

79. Yuzefovych L, Wilson G, Rachek L. Different effects of oleate vs. palmitate on mitochondrial function, apoptosis, and insulin signaling in L6 skeletal muscle cells: role of oxidative stress. Am J Physiol Endocrinol Metab 2010;299(6):E1096-105. doi: 10.1152/ajpendo.00238.2010.

80. Corcoran MP, Lamon-Fava S, Fielding RA. Skeletal muscle lipid deposition and insulin resistance: effect of dietary fatty acids and exercise. Am J Clin Nutr 2007;85(3):662-77.

81. Vessby B, Uusitupa M, Hermansen K, Riccardi G, Rivellese AA, Tapsell LC, Nalsen C, Berglund L, Louheranta A, Rasmussen BM, et al. Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. Diabetologia 2001;44(3):312-9.

82. Tierney AC, McMonagle J, Shaw DI, Gulseth HL, Helal O, Saris WH, Paniagua JA, Golabek-Leszczynska I, Defoort C, Williams CM, et al. Effects of dietary fat modification on insulin sensitivity and on other risk factors of the metabolic syndrome--LIPGENE: a European randomized dietary intervention study. Int J Obes (Lond) 2011;35(6):800-9. doi: 10.1038/ijo.2010.209.

83. Delarue J, LeFoll C, Corporeau C, Lucas D. N-3 long chain polyunsaturated fatty acids: a nutritional tool to prevent insulin resistance associated to type 2 diabetes and obesity? Reprod Nutr Dev 2004;44(3):289-99.

84. Soares MJ, Cummings SJ, Mamo JC, Kenrick M, Piers LS. The acute effects of olive oil v. cream on postprandial thermogenesis and substrate oxidation in postmenopausal women. Br J Nutr 2004;91(2):245-52. doi: 10.1079/bjn20031047.

85. Piers LS, Walker KZ, Stoney RM, Soares MJ, O'Dea K. The influence of the type of dietary fat on postprandial fat oxidation rates: monounsaturated (olive oil) vs saturated fat (cream). Int J Obes Relat Metab Disord 2002;26(6):814-21. doi: 10.1038/sj.ijo.0801993.

86. Kien CL, Bunn JY, Ugrasbul F. Increasing dietary palmitic acid decreases fat oxidation and daily energy expenditure. Am J Clin Nutr 2005;82(2):320-6.

87. Jones PJ, Jew S, AbuMweis S. The effect of dietary oleic, linoleic, and linolenic acids on fat oxidation and energy expenditure in healthy men. Metabolism 2008;57(9):1198-203. doi: 10.1016/j.metabol.2008.04.012.

88. Kien CL, Bunn JY, Tompkins CL, Dumas JA, Crain KI, Ebenstein DB, Koves TR, Muoio DM. Substituting dietary monounsaturated fat for saturated fat is associated with increased daily physical activity and resting energy expenditure and with changes in mood. Am J Clin Nutr 2013;97(4):689-97. doi: 10.3945/ajcn.112.051730.

89. Lovejoy JC, Smith SR, Champagne CM, Most MM, Lefevre M, DeLany JP, Denkins YM, Rood JC, Veldhuis J, Bray GA. Effects of diets enriched in saturated (palmitic), monounsaturated (oleic), or trans (elaidic) fatty acids on insulin sensitivity and substrate oxidation in healthy adults. Diabetes Care 2002;25(8):1283-8.

90. Casas-Agustench P, Lopez-Uriarte P, Bullo M, Ros E, Gomez-Flores A, Salas-Salvado J. Acute effects of three high-fat meals with different fat saturations on energy expenditure, substrate oxidation and satiety. Clin Nutr 2009;28(1):39-45. doi: 10.1016/j.clnu.2008.10.008.

91. Clevenger HC, Kozimor AL, Paton CM, Cooper JA. Acute effect of dietary fatty acid composition on postprandial metabolism in women. Exp Physiol 2014;99(9):1182-90. doi: 10.1113/expphysiol.2013.077222.

92. van Marken Lichtenbelt WD, Mensink RP, Westerterp KR. The effect of fat composition of the diet on energy metabolism. Z Ernahrungswiss 1997;36(4):303-5.

93. Rasmussen LG, Larsen TM, Mortensen PK, Due A, Astrup A. Effect on 24-h energy expenditure of a moderate-fat diet high in monounsaturated fatty acids compared with that of a low-fat, carbohydrate-rich diet: a 6-mo controlled dietary intervention trial. Am J Clin Nutr 2007;85(4):1014-22.

94. Maljaars J, Romeyn EA, Haddeman E, Peters HP, Masclee AA. Effect of fat saturation on satiety, hormone release, and food intake. Am J Clin Nutr 2009;89(4):1019-24. doi: 10.3945/ajcn.2008.27335.

95. Strik CM, Lithander FE, McGill AT, MacGibbon AK, McArdle BH, Poppitt SD. No evidence of differential effects of SFA, MUFA or PUFA on post-ingestive satiety and energy

intake: a randomised trial of fatty acid saturation. Nutr J 2010;9:24. doi: 10.1186/1475-2891-9-24.

96. Lawton CL, Delargy HJ, Brockman J, Smith FC, Blundell JE. The degree of saturation of fatty acids influences post-ingestive satiety. Br J Nutr 2000;83(5):473-82.

97. Romano A, Coccurello R, Giacovazzo G, Bedse G, Moles A, Gaetani S. Oleoylethanolamide: a novel potential pharmacological alternative to cannabinoid antagonists for the control of appetite. Biomed Res Int 2014;2014:203425. doi: 10.1155/2014/203425.

98. Simopoulos AP. An Increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. Nutrients 2016;8(3):128. doi: 10.3390/nu8030128.

99. Kim J, Carlson ME, Kuchel GA, Newman JW, Watkins BA. Dietary DHA reduces downstream endocannabinoid and inflammatory gene expression and epididymal fat mass while improving aspects of glucose use in muscle in C57BL/6J mice. Int J Obes (Lond) 2016;40(1):129-37. doi: 10.1038/ijo.2015.135.

100. Bowen KJ, Kris-Etherton PM, Shearer GC, West SG, Reddivari L, Jones PJ. Oleic acid-derived oleoylethanolamide: A nutritional science perspective. Prog Lipid Res 2017. doi: 10.1016/j.plipres.2017.04.001.

101. Nieters A, Becker N, Linseisen J. Polymorphisms in candidate obesity genes and their interaction with dietary intake of n-6 polyunsaturated fatty acids affect obesity risk in a sub-sample of the EPIC-Heidelberg cohort. Eur J Nutr 2002;41(5):210-21. doi: 10.1007/s00394-002-0378-y.

102. Larsen SC, Angquist L, Ostergaard JN, Ahluwalia TS, Vimaleswaran KS, Roswall N, Mortensen LM, Nielsen BM, Tjonneland A, Wareham NJ, et al. Intake of total and subgroups of fat minimally affect the associations between selected single nucleotide polymorphisms in the PPARgamma pathway and changes in anthropometry among European adults from cohorts of the DiOGenes study. J Nutr 2016;146(3):603-11. doi: 10.3945/jn.115.219675.

103. Rakhshandehroo M, Knoch B, Muller M, Kersten S. Peroxisome proliferator-activated receptor alpha target genes. PPAR Res 2010;2010. doi: 10.1155/2010/612089.

104. Ahmadian M, Suh JM, Hah N, Liddle C, Atkins AR, Downes M, Evans RM. PPARgamma signaling and metabolism: the good, the bad and the future. Nat Med 2013;19(5):557-66. doi: 10.1038/nm.3159.

105. Wang YX, Lee CH, Tiep S, Yu RT, Ham J, Kang H, Evans RM. Peroxisome-proliferator-activated receptor delta activates fat metabolism to prevent obesity. Cell 2003;113(2):159-70.

106. Larter CZ, Yeh MM, Cheng J, Williams J, Brown S, dela Pena A, Bell-Anderson KS, Farrell GC. Activation of peroxisome proliferator-activated receptor alpha by dietary fish oil attenuates steatosis, but does not prevent experimental steatohepatitis because of hepatic lipoperoxide accumulation. J Gastroenterol Hepatol 2008;23(2):267-75. doi: 10.1111/j.1440-1746.2007.05157.x.

107. Shiomi Y, Yamauchi T, Iwabu M, Okada-Iwabu M, Nakayama R, Orikawa Y, Yoshioka Y, Tanaka K, Ueki K, Kadowaki T. A Novel peroxisome proliferator-activated receptor

(PPAR)alpha agonist and PPARgamma antagonist, Z-551, ameliorates high-fat diet-induced obesity and metabolic disorders in mice. J Biol Chem 2015;290(23):14567-81. doi: 10.1074/jbc.M114.622191.

108. Jeong S, Yoon M. Fenofibrate inhibits adipocyte hypertrophy and insulin resistance by activating adipose PPARalpha in high fat diet-induced obese mice. Exp Mol Med 2009;41(6):397-405. doi: 10.3858/emm.2009.41.6.045.

109. Laplante M, Sell H, MacNaul KL, Richard D, Berger JP, Deshaies Y. PPAR-gamma activation mediates adipose depot-specific effects on gene expression and lipoprotein lipase activity: mechanisms for modulation of postprandial lipemia and differential adipose accretion. Diabetes 2003;52(2):291-9.

110. Festuccia WT, Blanchard PG, Turcotte V, Laplante M, Sariahmetoglu M, Brindley DN, Deshaies Y. Depot-specific effects of the PPARgamma agonist rosiglitazone on adipose tissue glucose uptake and metabolism. J Lipid Res 2009;50(6):1185-94. doi: 10.1194/jlr.M800620-JLR200.

111. Luan J, Browne PO, Harding AH, Halsall DJ, O'Rahilly S, Chatterjee VK, Wareham NJ. Evidence for gene-nutrient interaction at the PPARgamma locus. Diabetes 2001;50(3):686-9.

112. Memisoglu A, Hu FB, Hankinson SE, Manson JE, De Vivo I, Willett WC, Hunter DJ. Interaction between a peroxisome proliferator-activated receptor gamma gene polymorphism and dietary fat intake in relation to body mass. Hum Mol Genet 2003;12(22):2923-9. doi: 10.1093/hmg/ddg318.

113. Kersten S. Mechanisms of nutritional and hormonal regulation of lipogenesis. EMBO Rep 2001;2(4):282-6. doi: 10.1093/embo-reports/kve071.

114. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest 2002;109(9):1125-31. doi: 10.1172/jci15593.

115. Shimano H. Sterol regulatory element-binding proteins (SREBPs): transcriptional regulators of lipid synthetic genes. Prog Lipid Res 2001;40(6):439-52.

116. Vara Prasad SS, Jeya Kumar SS, Kumar PU, Qadri SS, Vajreswari A. Dietary fatty acid composition alters 11beta-hydroxysteroid dehydrogenase type 1 gene expression in rat retroperitoneal white adipose tissue. Lipids Health Dis 2010;9:111. doi: 10.1186/1476-511x-9-111.

117. Clarke SD. Polyunsaturated fatty acid regulation of gene transcription: a mechanism to improve energy balance and insulin resistance. Br J Nutr 2000;83 Suppl 1:S59-66.

118. Reilly JM, Thompson MP. Dietary fatty acids up-regulate the expression of UCP2 in 3T3-L1 preadipocytes. Biochem Biophys Res Commun 2000;277(3):541-5. doi: 10.1006/bbrc.2000.3705.

119. Clarke SD, Turini M, Jump DB, Abraham S, Reedy M. Polyunsaturated fatty acid inhibition of fatty acid synthase transcription is independent of PPAR activation. Z Ernahrungswiss 1998;37 Suppl 1:14-20.

120. Mejia-Barradas CM, Del-Rio-Navarro BE, Dominguez-Lopez A, Campos-Rodriguez R, Martinez-Godinez M, Rojas-Hernandez S, Lara-Padilla E, Abarca-Rojano E, Miliar-Garcia A. The consumption of n-3 polyunsaturated fatty acids differentially modulates gene expression of peroxisome proliferator-activated receptor alpha and gamma and hypoxia-inducible factor 1 alpha in subcutaneous adipose tissue of obese adolescents. Endocrine 2014;45(1):98-105. doi: 10.1007/s12020-013-9941-y.

121. Naughton SS, Mathai ML, Hryciw DH, McAinch AJ. Linoleic acid and the pathogenesis of obesity. Prostaglandins Other Lipid Mediat 2016;125:90-9. doi: 10.1016/j.prostaglandins.2016.06.003.

122. Meyer BJ, Mann NJ, Lewis JL, Milligan GC, Sinclair AJ, Howe PR. Dietary intakes and food sources of omega-6 and omega-3 polyunsaturated fatty acids. Lipids 2003;38(4):391-8.

123. Ruzickova J, Rossmeisl M, Prazak T, Flachs P, Sponarova J, Veck M, Tvrzicka E, Bryhn M, Kopecky J. Omega-3 PUFA of marine origin limit diet-induced obesity in mice by reducing cellularity of adipose tissue. Lipids 2004;39(12):1177-85.

124. Lands WE, Morris A, Libelt B. Quantitative effects of dietary polyunsaturated fats on the composition of fatty acids in rat tissues. Lipids 1990;25(9):505-16.

125. Bueno AA, Oyama LM, de Oliveira C, Pisani LP, Ribeiro EB, Silveira VL, Oller do Nascimento CM. Effects of different fatty acids and dietary lipids on adiponectin gene expression in 3T3-L1 cells and C57BL/6J mice adipose tissue. Pflugers Arch 2008;455(4):701-9. doi: 10.1007/s00424-007-0330-3.

126. Yang ZH, Miyahara H, Iwasaki Y, Takeo J, Katayama M. Dietary supplementation with long-chain monounsaturated fatty acids attenuates obesity-related metabolic dysfunction and increases expression of PPAR gamma in adipose tissue in type 2 diabetic KK-Ay mice. Nutr Metab (Lond) 2013;10(1):16. doi: 10.1186/1743-7075-10-16.

127. Hernando Boigues JF, Mach N. The effect of polyunsaturated fatty acids on obesity through epigenetic modifications. Endocrinol Nutr 2015;62(7):338-49. doi: 10.1016/j.endonu.2015.03.009.

128. Ceccarelli V, Racanicchi S, Martelli MP, Nocentini G, Fettucciari K, Riccardi C, Marconi P, Di Nardo P, Grignani F, Binaglia L, et al. Eicosapentaenoic acid demethylates a single CpG that mediates expression of tumor suppressor CCAAT/enhancer-binding protein delta in U937 leukemia cells. J Biol Chem 2011;286(31):27092-102. doi: 10.1074/jbc.M111.253609.

129. Palmer DJ, Huang RC, Craig JM, Prescott SL. Nutritional influences on epigenetic programming: asthma, allergy, and obesity. Immunol Allergy Clin North Am 2014;34(4):825-37. doi: 10.1016/j.iac.2014.07.003.

130. Voisin S, Almen MS, Moschonis G, Chrousos GP, Manios Y, Schioth HB. Dietary fat quality impacts genome-wide DNA methylation patterns in a cross-sectional study of Greek preadolescents. Eur J Hum Genet 2015;23(5):654-62. doi: 10.1038/ejhg.2014.139.

131. Hall E, Volkov P, Dayeh T, Bacos K, Ronn T, Nitert MD, Ling C. Effects of palmitate on genome-wide mRNA expression and DNA methylation patterns in human pancreatic islets. BMC Med 2014;12:103. doi: 10.1186/1741-7015-12-103.

132. Silva-Martinez GA, Rodriguez-Rios D, Alvarado-Caudillo Y, Vaquero A, Esteller M, Carmona FJ, Moran S, Nielsen FC, Wickstrom-Lindholm M, Wrobel K, et al. Arachidonic and oleic acid exert distinct effects on the DNA methylome. Epigenetics 2016;11(5):321-34. doi: 10.1080/15592294.2016.1161873.

133. Perfilyev A, Dahlman I, Gillberg L, Rosqvist F, Iggman D, Volkov P, Nilsson E, Riserus U, Ling C. Impact of polyunsaturated and saturated fat overfeeding on the DNA-methylation pattern in human adipose tissue: a randomized controlled trial. Am J Clin Nutr 2017;105(4):991-1000. doi: 10.3945/ajcn.116.143164.

134. Shen W, Wang C, Xia L, Fan C, Dong H, Deckelbaum RJ, Qi K. Epigenetic modification of the leptin promoter in diet-induced obese mice and the effects of N-3 polyunsaturated fatty acids. Sci Rep 2014;4:5282. doi: 10.1038/srep05282.

135. Corella D, Arnett DK, Tucker KL, Kabagambe EK, Tsai M, Parnell LD, Lai CQ, Lee YC, Warodomwichit D, Hopkins PN, et al. A high intake of saturated fatty acids strengthens the association between the fat mass and obesity-associated gene and BMI. J Nutr 2011;141(12):2219-25. doi: 10.3945/jn.111.143826.

136. Chouinard-Watkins R, Conway V, Minihane AM, Jackson KG, Lovegrove JA, Plourde M. Interaction between BMI and APOE genotype is associated with changes in the plasma long-chain-PUFA response to a fish-oil supplement in healthy participants. Am J Clin Nutr 2015;102(2):505-13. doi: 10.3945/ajcn.114.103507.

137. Robitaille J, Brouillette C, Lemieux S, Perusse L, Gaudet D, Vohl MC. Plasma concentrations of apolipoprotein B are modulated by a gene--diet interaction effect between the LFABP T94A polymorphism and dietary fat intake in French-Canadian men. Mol Genet Metab 2004;82(4):296-303. doi: 10.1016/j.ymgme.2004.06.002.

138. Ma Y, Tucker KL, Smith CE, Lee YC, Huang T, Richardson K, Parnell LD, Lai CQ, Young KL, Justice AE, et al. Lipoprotein lipase variants interact with polyunsaturated fatty acids for obesity traits in women: replication in two populations. Nutr Metab Cardiovasc Dis 2014;24(12):1323-9. doi: 10.1016/j.numecd.2014.07.003.

139. Smith CE, Arnett DK, Corella D, Tsai MY, Lai CQ, Parnell LD, Lee YC, Ordovas JM. Perilipin polymorphism interacts with saturated fat and carbohydrates to modulate insulin resistance. Nutr Metab Cardiovasc Dis 2012;22(5):449-55. doi: 10.1016/j.numecd.2010.09.003.

140. Garaulet M, Smith CE, Hernandez-Gonzalez T, Lee YC, Ordovas JM. PPARgamma Pro12Ala interacts with fat intake for obesity and weight loss in a behavioural treatment based on the Mediterranean diet. Mol Nutr Food Res 2011;55(12):1771-9. doi: 10.1002/mnfr.201100437.

141. Phillips CM, Kesse-Guyot E, McManus R, Hercberg S, Lairon D, Planells R, Roche HM. High dietary saturated fat intake accentuates obesity risk associated with the fat mass and obesity-associated gene in adults. J Nutr 2012;142(5):824-31. doi: 10.3945/jn.111.153460.

142. Wiklund P, Toss F, Weinehall L, Hallmans G, Franks PW, Nordstrom A, Nordstrom P. Abdominal and gynoid fat mass are associated with cardiovascular risk factors in men and women. J Clin Endocrinol Metab 2008;93(11):4360-6. doi: 10.1210/jc.2008-0804.

143. Ibrahim MM. Subcutaneous and visceral adipose tissue: structural and functional differences. Obes Rev 2010;11(1):11-8. doi: 10.1111/j.1467-789X.2009.00623.x.

144. Rothney MP, Catapano AL, Xia J, Wacker WK, Tidone C, Grigore L, Xia Y, Ergun DL. Abdominal visceral fat measurement using dual-energy X-ray: association with cardiometabolic risk factors. Obesity (Silver Spring) 2013;21(9):1798-802. doi: 10.1002/oby.20223.

145. Snijder MB, Visser M, Dekker JM, Seidell JC, Fuerst T, Tylavsky F, Cauley J, Lang T, Nevitt M, Harris TB. The prediction of visceral fat by dual-energy X-ray absorptiometry in the elderly: a comparison with computed tomography and anthropometry. Int J Obes Relat Metab Disord 2002;26(7):984-93. doi: 10.1038/sj.ijo.0801968.

146. Kaul S, Rothney MP, Peters DM, Wacker WK, Davis CE, Shapiro MD, Ergun DL. Dual-energy X-ray absorptiometry for quantification of visceral fat. Obesity (Silver Spring) 2012;20(6):1313-8. doi: 10.1038/oby.2011.393.

147. Miazgowski T, Krzyzanowska-Swiniarska B, Dziwura-Ogonowska J, Widecka K. The associations between cardiometabolic risk factors and visceral fat measured by a new dual-energy X-ray absorptiometry-derived method in lean healthy Caucasian women. Endocrine 2014;47(2):500-5. doi: 10.1007/s12020-014-0180-7.

148. Gealekman O, Guseva N, Hartigan C, Apotheker S, Gorgoglione M, Gurav K, Tran KV, Straubhaar J, Nicoloro S, Czech MP, et al. Depot-specific differences and insufficient subcutaneous adipose tissue angiogenesis in human obesity. Circulation 2011;123(2):186-94. doi: 10.1161/circulationaha.110.970145.

149. Chaston TB, Dixon JB. Factors associated with percent change in visceral versus subcutaneous abdominal fat during weight loss: findings from a systematic review. Int J Obes (Lond) 2008;32(4):619-28. doi: 10.1038/sj.ijo.0803761.

150. Caron-Jobin M, Mauvoisin D, Michaud A, Veilleux A, Noel S, Fortier MP, Julien P, Tchernof A, Mounier C. Stearic acid content of abdominal adipose tissues in obese women. Nutr Diabetes 2012;2:e23. doi: 10.1038/nutd.2011.19.

151. Davidge-Pitts C, Escande CJ, Conover CA. Preferential expression of PAPPA in human preadipocytes from omental fat. J Endocrinol 2014;222(1):87-97. doi: 10.1530/joe-13-0610.

152. Lefebvre AM, Laville M, Vega N, Riou JP, van Gaal L, Auwerx J, Vidal H. Depot-specific differences in adipose tissue gene expression in lean and obese subjects. Diabetes 1998;47(1):98-103.

153. Guiu-Jurado E, Auguet T, Berlanga A, Aragones G, Aguilar C, Sabench F, Armengol S, Porras JA, Marti A, Jorba R, et al. Downregulation of de novo fatty acid synthesis in subcutaneous adipose tissue of moderately obese women. Int J Mol Sci 2015;16(12):29911-22. doi: 10.3390/ijms161226206.

154. Ray H, Pinteur C, Frering V, Beylot M, Large V. Depot-specific differences in perilipin and hormone-sensitive lipase expression in lean and obese. Lipids Health Dis 2009;8:58. doi: 10.1186/1476-511x-8-58.

155. Huang ZH, Espiritu DJ, Uy A, Holterman AX, Vitello J, Mazzone T. Adipose tissue depot-specific differences in adipocyte apolipoprotein E expression. Metabolism 2011;60(12):1692-701. doi: 10.1016/j.metabol.2011.04.012.

156. Ramanjaneya M, Chen J, Brown JE, Tripathi G, Hallschmid M, Patel S, Kern W, Hillhouse EW, Lehnert H, Tan BK, et al. Identification of nesfatin-1 in human and murine adipose tissue: a novel depot-specific adipokine with increased levels in obesity. Endocrinology 2010;151(7):3169-80. doi: 10.1210/en.2009-1358.

157. Raclot T, Oudart H. Selectivity of fatty acids on lipid metabolism and gene expression. Proc Nutr Soc 1999;58(3):633-46.

158. Robitaille J, Despres JP, Perusse L, Vohl MC. The PPAR-gamma P12A polymorphism modulates the relationship between dietary fat intake and components of the metabolic syndrome: results from the Quebec Family Study. Clin Genet 2003;63(2):109-16.

159. Rosado EL, Bressan J, Martinez JA, Marques-Lopes I. Interactions of the PPARgamma2 polymorphism with fat intake affecting energy metabolism and nutritional outcomes in obese women. Ann Nutr Metab 2010;57(3-4):242-50. doi: 10.1159/000322187.

160. Franks PW, Jablonski KA, Delahanty L, Hanson RL, Kahn SE, Altshuler D, Knowler WC, Florez JC. The Pro12Ala variant at the peroxisome proliferator-activated receptor gamma gene and change in obesity-related traits in the Diabetes Prevention Program. Diabetologia 2007;50(12):2451-60. doi: 10.1007/s00125-007-0826-6.

161. Rosado EL, Bressan J, Hernandez JA, Martins MF, Cecon PR. [Effect of diet and PPARgamma2 and beta2-adrenergic receptor genes on energy metabolism and body composition in obese women]. Nutr Hosp 2006;21(3):317-31.

162. de Luis DA, Aller R, Izaola O, Conde R, Eiros Bouza JM. Genetic variation in the beta 3-adrenoreceptor gene (Trp64Arg polymorphism) and its influence on anthropometric parameters and insulin resistance under a high monounsaturated versus a high polyunsaturated fat hypocaloric diet. Ann Nutr Metab 2013;62(4):303-9. doi: 10.1159/000346800.

163. Razquin C, Martinez JA, Martinez-Gonzalez MA, Bes-Rastrollo M, Fernandez-Crehuet J, Marti A. A 3-year intervention with a Mediterranean diet modified the association between the rs9939609 gene variant in FTO and body weight changes. Int J Obes (Lond) 2010;34(2):266-72. doi: 10.1038/ijo.2009.233.

164. De Luis DA, Aller R, Izaola O, Pacheco D. Role of rs9939609 FTO gene variant in weight loss, insulin resistance and metabolic parameters after a high monounsaturated vs a high polyunsaturated fat hypocaloric diets. Nutr Hosp 2015;32(1):175-81. doi: 10.3305/nh.2015.32.1.9169.

165. Dominguez-Reyes T, Astudillo-Lopez CC, Salgado-Goytia L, Munoz-Valle JF, Salgado-Bernabe AB, Guzman-Guzman IP, Castro-Alarcon N, Moreno-Godinez ME, Parra-Rojas I. Interaction of dietary fat intake with APOA2, APOA5 and LEPR polymorphisms and its

relationship with obesity and dyslipidemia in young subjects. Lipids Health Dis 2015;14:106. doi: 10.1186/s12944-015-0112-4.

166. Warodomwichit D, Shen J, Arnett DK, Tsai MY, Kabagambe EK, Peacock JM, Hixson JE, Straka RJ, Province MA, An P, et al. ADIPOQ polymorphisms, monounsaturated fatty acids, and obesity risk: the GOLDN study. Obesity (Silver Spring) 2009;17(3):510-7. doi: 10.1038/oby.2008.583.

167. de Luis DA, Aller R, Izaola O, Bachiller R, Pacheco D. Cardiovascular risk factors and adipocytokines levels after two hypocaloric diets with different fat distribution in obese subjects and rs6923761 gene variant of glucagon-like peptide 1 receptor. J Endocrinol Invest 2014;37(9):853-9. doi: 10.1007/s40618-014-0116-3.

168. Garaulet M, Lee YC, Shen J, Parnell LD, Arnett DK, Tsai MY, Lai CQ, Ordovas JM. CLOCK genetic variation and metabolic syndrome risk: modulation by monounsaturated fatty acids. Am J Clin Nutr 2009;90(6):1466-75. doi: 10.3945/ajcn.2009.27536.

169. de Luis DA, Aller R, Gonzalez Sagrado M, Conde R, Izaola O, de la Fuente B. Genetic variation in the cannabinoid receptor gene (CNR1) (G1359A polymorphism) and their influence on anthropometric parameters and metabolic parameters under a high monounsaturated vs. high polyunsaturated fat hypocaloric diets. J Nutr Biochem 2013;24(8):1431-5. doi: 10.1016/j.jnutbio.2012.12.001.

170. Ma XY, Qiu WQ, Smith CE, Parnell LD, Jiang ZY, Ordovas JM, Tucker KL, Lai CQ. Association between BDNF rs6265 and obesity in the Boston Puerto Rican Health Study. J Obes 2012;2012:102942. doi: 10.1155/2012/102942.

171. Garaulet M, Smith CE, Gomez-Abellan P, Ordovas-Montanes M, Lee YC, Parnell LD, Arnett DK, Ordovas JM. REV-ERB-ALPHA circadian gene variant associates with obesity in two independent populations: Mediterranean and North American. Mol Nutr Food Res 2014;58(4):821-9. doi: 10.1002/mnfr.201300361.

172. Phillips CM, Goumidi L, Bertrais S, Field MR, Peloso GM, Shen J, McManus R, Hercberg S, Lairon D, Planells R, et al. Dietary saturated fat modulates the association between STAT3 polymorphisms and abdominal obesity in adults. J Nutr 2009;139(11):2011-7. doi: 10.3945/jn.109.110635.

173. Joffe YT, van der Merwe L, Evans J, Collins M, Lambert EV, September AV, Goedecke JH. Interleukin-6 gene polymorphisms, dietary fat intake, obesity and serum lipid concentrations in black and white South African women. Nutrients 2014;6(6):2436-65. doi: 10.3390/nu6062436.

174. Razquin C, Martinez JA, Martinez-Gonzalez MA, Fernandez-Crehuet J, Santos JM, Marti A. A Mediterranean diet rich in virgin olive oil may reverse the effects of the -174G/C IL6 gene variant on 3-year body weight change. Mol Nutr Food Res 2010;54 Suppl 1:S75-82. doi: 10.1002/mnfr.200900257.

175. Joffe YT, van der Merwe L, Evans J, Collins M, Lambert EV, September A, Goedecke JH. The tumor necrosis factor-alpha gene -238G>A polymorphism, dietary fat intake, obesity risk and serum lipid concentrations in black and white South African women. Eur J Clin Nutr 2012;66(12):1295-302. doi: 10.1038/ejcn.2012.156.

176. Joffe YT, van der Merwe L, Carstens M, Collins M, Jennings C, Levitt NS, Lambert EV, Goedecke JH. Tumor necrosis factor-alpha gene -308 G/A polymorphism modulates the relationship between dietary fat intake, serum lipids, and obesity risk in black South African women. J Nutr 2010;140(5):901-7. doi: 10.3945/jn.109.109355.

177. de Luis DA, Aller R, Izaola O, Gonzalez Sagrado M, Conde R. Role of G308 promoter variant of tumor necrosis factor alpha gene on weight loss and metabolic parameters after a high monounsaturated versus a high polyunsaturated fat hypocaloric diets. Med Clin (Barc) 2013;141(5):189-93. doi: 10.1016/j.medcli.2012.12.021.

178. Junyent M, Parnell LD, Lai CQ, Arnett DK, Tsai MY, Kabagambe EK, Straka RJ, Province M, An P, Smith CE, et al. ADAM17_i33708A>G polymorphism interacts with dietary n-6 polyunsaturated fatty acids to modulate obesity risk in the Genetics of Lipid Lowering Drugs and Diet Network study. Nutr Metab Cardiovasc Dis 2010;20(10):698-705. doi: 10.1016/j.numecd.2009.06.011.

179. Corella D, Lai CQ, Demissie S, Cupples LA, Manning AK, Tucker KL, Ordovas JM. APOA5 gene variation modulates the effects of dietary fat intake on body mass index and obesity risk in the Framingham Heart Study. J Mol Med (Berl) 2007;85(2):119-28. doi: 10.1007/s00109-006-0147-0.

180. Corella D, Peloso G, Arnett DK, Demissie S, Cupples LA, Tucker K, Lai CQ, Parnell LD, Coltell O, Lee YC, et al. APOA2, dietary fat, and body mass index: replication of a gene-diet interaction in 3 independent populations. Arch Intern Med 2009;169(20):1897-906. doi: 10.1001/archinternmed.2009.343.

181. Smith CE, Tucker KL, Arnett DK, Noel SE, Corella D, Borecki IB, Feitosa MF, Aslibekyan S, Parnell LD, Lai CQ, et al. Apolipoprotein A2 polymorphism interacts with intakes of dairy foods to influence body weight in 2 U.S. populations. J Nutr 2013;143(12):1865-71. doi: 10.3945/jn.113.179051.

182. Basiri MG, Sotoudeh G, Alvandi E, Djalali M, Eshraghian MR, Noorshahi N, Koohdani F. APOA2 -256T>C polymorphism interacts with saturated fatty acids intake to affect anthropometric and hormonal variables in type 2 diabetic patients. Genes Nutr 2015;10(3):464. doi: 10.1007/s12263-015-0464-4.

183. Smith CE, Ordovas JM, Sanchez-Moreno C, Lee YC, Garaulet M. Apolipoprotein A-II polymorphism: relationships to behavioural and hormonal mediators of obesity. Int J Obes (Lond) 2012;36(1):130-6. doi: 10.1038/ijo.2011.24.

184. Corella D, Tai ES, Sorli JV, Chew SK, Coltell O, Sotos-Prieto M, Garcia-Rios A, Estruch R, Ordovas JM. Association between the APOA2 promoter polymorphism and body weight in Mediterranean and Asian populations: replication of a gene-saturated fat interaction. Int J Obes (Lond) 2011;35(5):666-75. doi: 10.1038/ijo.2010.187.

185. Rafiee M, Sotoudeh G, Djalali M, Alvandi E, Eshraghian M, Sojoudi F, Koohdani F. Dietary omega-3 polyunsaturated fatty acid intake modulates impact of Insertion/Deletion polymorphism of ApoB gene on obesity risk in type 2 diabetic patients. Nutrition 2016;32(10):1110-5. doi: 10.1016/j.nut.2016.03.012.

186. Smith CE, Tucker KL, Lee YC, Lai CQ, Parnell LD, Ordovas JM. Low-density lipoprotein receptor-related protein 1 variant interacts with saturated fatty acids in Puerto Ricans. Obesity (Silver Spring) 2013;21(3):602-8. doi: 10.1002/oby.20001.

187. Smith CE, Ngwa J, Tanaka T, Qi Q, Wojczynski MK, Lemaitre RN, Anderson JS, Manichaikul A, Mikkila V, van Rooij FJ, et al. Lipoprotein receptor-related protein 1 variants and dietary fatty acids: meta-analysis of European origin and African American studies. Int J Obes (Lond) 2013;37(9):1211-20. doi: 10.1038/ijo.2012.215.

BRIDGE TO CHAPTER III

Chapter II has highlighted the beneficial influence of dietary MUFA on modulating obesity and interacting with genes in lipogenesis and adipogenesis pathways to modify fat accumulation. However, a limited number of genetic polymorphisms have been investigated, thus far, regarding their effect in altering the responsiveness of fat mass accumulation and distribution to MUFA consumption. Numerous polymorphisms in lipid metabolism-related genes are yet to be investigated, therefore, Chapter III comprises a manuscript that evaluates a potential role of 21 candidate common variants of genes along obesogenic pathways in the responsiveness of fat mass changes to different levels of dietary MUFA. Shatha Hammad participated in study coordination and data collection at Richardson Centre for Functional Food and Nutraceuticals (RCFFN), processed body composition data for all participants, conducted gentyping analyses, performed the statistical analyses, and wrote the manuscript.

CHAPTER III

MANUSCRIPT 2

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Common variants in lipid metabolism–related genes associate with fat mass changes in response to dietary monounsaturated fatty acids

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3.1 Abstract

Background: The global prevalence of obesity is increasing rapidly. Different fatty acids may vary in their obesogenic effect, and the genetic makeup may contribute to fat deposition in response to dietary fatty acid composition. However, the obesogenic effects of the interactions between dietary monounsaturated fatty acids (MUFA) and genetic factors have been scarcely tested in intervention studies.

Objective: We evaluated the changes in body weight and body fat mass distribution in response to MUFA consumption, as well as the associations of common genetic variants in lipid metabolism-related genes on this response.

Design: Adults (n=115) with abdominal obesity were included in a randomized, crossover, iso-caloric, controlled-feeding trial. Participants consumed one of three oils (20% of total energy) for six weeks, separated by a four-twelve week washout. Oils included two-high-MUFA oils, conventional canola and high-oleic canola, and one low-MUFA high-saturated fatty acid (SFA) oil blend. Dual Energy X-ray Absorptiometry scans were used to measure total and regional body fat mass. Genotyping of 21 candidate single-nucleotide polymorphisms (SNPs) was performed using qualitative PCR.

Results: No significant differences were observed in changes in body weight or fat mass following the consumption of either high-MUFA diet compared to the low-MUFA high-SFA treatment. However, when stratified by the genotype, seven SNPs influenced the distributions of fat mass in response to treatment (n=101). The consumption of high-MUFA diets induced significant reductions in various regions of fat mass in the *LPL* rs13702-CC, *PPARα* rs6008259-AA, and *ADIPOQ* rs266729-GG carriers. Moreover, the low-MUFA high-SFA diet promoted fat mass

reductions in the *FTO* rs9939609-A, *APOE4*, *ADRB2* rs1042714-GG, and *LIPC* rs6083-GG carriers.

Conclusion: Common variants in *LPL*, *FTO*, *PPARα*, *ADIPOQ*, *APOE*, *LIPC*, and *ADRB2* genes modulated body fat mass distribution in response to different levels of dietary MUFA in an iso-caloric diet. These findings may eventually help in developing personalized dietary recommendations.

3.2 Introduction

Dietary fatty acid (FA) composition has been recognized as a determinant of fat deposition and distribution (1-4). Various FAs may vary in their obesity-inducing effects by influencing energy expenditure, fat oxidation, and thermogenesis, and/or modulating appetite sensation (5-7). An increasing body of evidence has demonstrated that dietary monounsaturated fatty acids (MUFA) increased fat oxidation, diet-induced thermogenesis (7-9), resting energy expenditure (10), and promoted weight loss (11, 12) to a greater extent compared to saturated fatty acids (SFA). Our recent controlled feeding study showed that two test diets high in MUFA, canola oil and a high-oleic canola oil, significantly reduced android fat mass compared with a high-polyunsaturated fatty acids (PUFA) flaxseed/safflower oil in participants with abdominal obesity (1). These favorable effects may be attributed, partly, to interactions between FAs and genetic polymorphisms.

The genetic contribution to obesity is well recognized and the heritability is estimated to contribute to 40-70% (13, 14). The responses of individuals with obesity to weight-gain prevention and reduction strategies may vary broadly based on their genetic makeup (15). Therefore, examining gene-nutrient interactions assists in estimating the role of qualitative FA intake on the onset/progression of obesity in a genotype-specific manner.

The evidence from controlled trials and observational studies suggests a contribution of gene-FA interactions in modulating adiposity via several mechanisms. For instance, the consumption of a higher proportion of MUFA (16-18) and PUFA (19, 20) relative to SFA was found to be associated with lower body weight in peroxisome proliferator activated receptor-gamma (*PPARγ*) rs1801282-G allele carriers in different ethnic populations. In contrast, a meta-analysis of 14 studies of US and European Caucasians revealed a direct association between SFA

consumption and body mass index (BMI) as well as waist-to-hip ratio (WHR) in the rs2306692-TT genotype carriers of low-density lipoprotein-related receptor protein-1 (21).

Although current research provides an emerging evidence for gene-MUFA interactions (16-18, 22-24), numerous polymorphisms in lipid metabolism-related genes are yet to be investigated. Moreover, to investigate fat deposition and distribution often times surrogate biomarkers were utilized, potentially masking outcomes. To meet our objective, we used whole-body Dual Energy X-ray Absorptiometry (DXA) scanning, which provides a reliable identification of fat distribution and discrimination between different fat depots, to assess associations of common genetic variants on changes of total and regional fat mass following a six-week controlled iso-caloric dietary intervention with different levels of MUFA.

3.3 Subjects and Methods

3.3.1 Study design and population

This study of gene-nutrient interactions was conducted within the framework of the Canola Oil Multi-center Intervention Trial II (COMIT II). COMIT II is a randomized, controlled, double-blind, crossover study designed to evaluate the response in body composition to three oils with different MUFA levels including regular canola oil (RCO), high-oleic acid canola oil (HOCO), and a low-MUFA high-SFA oil blend. This trial was conducted from 2014-2016 at three sites in Canada and one site in the United States: the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN) at the University of Manitoba in Winnipeg, the Canadian Centre for Agri-Food Research in Health and Medicine (CCARM) at St. Boniface Hospital Albrechtsen Research Centre in Winnipeg, the Institute of Nutrition and Functional Foods (INAF) at Laval University in Québec City, and the Departments of Nutritional Sciences and

Biobehavioral Health at The Pennsylvania State University in University Park (PSU). The protocol was reviewed and approved by institutional ethics boards across the participating clinical sites. The trial was registered at clinicaltrials.gov as NCT02029833.

Participants aged 20-65 years were included if they had abdominal obesity according to the International Diabetes Federation cutoff point for waist circumference (94 cm in men and 80 cm in women) in addition to at least one of the following metabolic syndrome criteria: fasting blood glucose of ≥ 5.6 mmol/L, triglycerides ≥1.7 mmol/L, high-density lipoprotein-cholesterol <1 mmol/L (men) or <1.3 mmol/L (women), and blood pressure ≥130 mmHg (systolic) and/or ≥85 mmHg (diastolic). Individuals were excluded if they had unstable thyroid disease, kidney disease, diabetes mellitus, or liver disease. Current smokers, individuals consuming > 14 alcoholic beverages per week, individuals taking medication known to affect lipid metabolism for at least the last three months, or individuals who were unwilling to stop taking any supplement at least two weeks before the study were not permitted to participate. Written informed consent was obtained from all participants upon enrollment. Participants were randomly assigned to one of six treatment sequences using a random number generator at randomization.com.

3.3.2 Study diets

This study consisted of three treatment periods during which the participants consumed a controlled iso-caloric, full-feeding diet with a fixed macronutrient composition of 35% fat, 50% carbohydrate, and 15% protein of total energy, as well as ~208 mg/3000 kcal/d cholesterol and ~38 g/3000 kcal/d fiber. Menus for the three phases were identical except for the type of treatment oil provided. Treatment phases extended for six weeks and were separated by six-week washout periods, the washout peroids ranged from four to twelve-week in some cases to meet the

participants needs. During the washout period, participants were instructed to consume their habitual diets. Participants were asked to maintain their usual level of planned and structured physical activity throughout the entire study. Physical activity changes were monitored by a weekly questionnaire.

The treatment oils which comprised of 20% of total energy were incorporated into a smoothie beverage and were equally divided into two portions consumed at breakfast and supper. Treatment oils included: 1) regular canola oil (Canola Harvest Canola Oil, Richardson International, Winnipeg, Canada) that provided 6.6% SFA, 65.3% MUFA, 19.6% n-6 PUFA, 8.5% n-3 PUFA α-linolenic acid, 2) high-oleic acid canola oil (Canola Harvest Canola Oil, Richardson International, Winnipeg, Canada) that provided 6.7% SFA, 75.9% MUFA, 14.8% n-6 PUFA, 2.6% n-3 PUFA α-linolenic acid, and 3) a low-MUFA high-SFA oil blend that provided 40.2% SFA, 22.0% MUFA, 29.6% n-6 PUFA, 8.2% n-3 PUFA α-linolenic acid. The low-MUFA high-SFA oil blend was prepared using commercially available ghee/butter oil (36.0%, Verka, New Delhi, Delhi, India), safflower oil (34.9%, eSutras, Illinois, Chicago, USA), coconut oil (16.0%, eSutras, Illinois, Chicago, USA), and flaxseed oil (13.1%, Shape Foods, Brandon, Canada). Study food and treatment shakes were prepared based on a seven-day rotating menu cycle in the metabolic kitchen of the participating sites. Compliance was assessed by smoothie consumption where the participants were required to consume at least 90% of the smoothies provided at each phase. Participants signed a daily checklist to verify smoothies' consumption. To maximize the compliance rate, participants were required to consume one smoothie at breakfast under the supervision of a clinical coordinator for five days/week. During weekdays, participants were provided the rest of their meals and a second smoothie in a food

cooler bag for consumption off-site. Weekend meals and treatment shakes were delivered to the participants' residence or handed out to them, upon their request, at the clinical site on Fridays.

3.3.3 Measurement of fat mass

DXA scans were performed by a trained operator using Lunar Prodigy Advance DXA (GE Healthcare, Madison, WI, USA) with the default configurations. A DXA scan was performed for all participants at the initiation and termination of each dietary phase. Participants were asked to remove any metal items and heavy clothes before scanning. Regions of interests were manually adjusted using enCORE 2012 software (version 14.10.022) according to the manufacturer's instructions. Fat mass was analyzed as total fat mass, as well as four different districts including trunk, legs, android, and gynoid fat mass. The android and gynoid regions of interest (ROI) were identified as per the manufacturer's instructions (**Figure 3.1**). The android region has been defined as a portion of the abdomen that starts at the pelvis cut line and extends upward to include 20% of distance between the pelvis and neck cut lines, with the outer arms cuts as the lateral boundaries. The gynoid region has been defined as a portion of the legs with upper boundary below the pelvis cut line by 1.5 times the height of the android region, it extends downward to two times the height of the android ROI, with the outer leg cuts as the lateral boundaries. Further, visceral adipose tissue (VAT) was assessed by the CoreScan feature in enCORE 2012 software (version 14.10.022), and used to calculate the subcutaneous adipose tissue (SCAT) by subtracting VAT mass from android fat mass (25). VAT measurement using the CoreScan has been validated using computed tomography scan (26). Criteria used to identify the anatomical region of interest were identical across all sites.

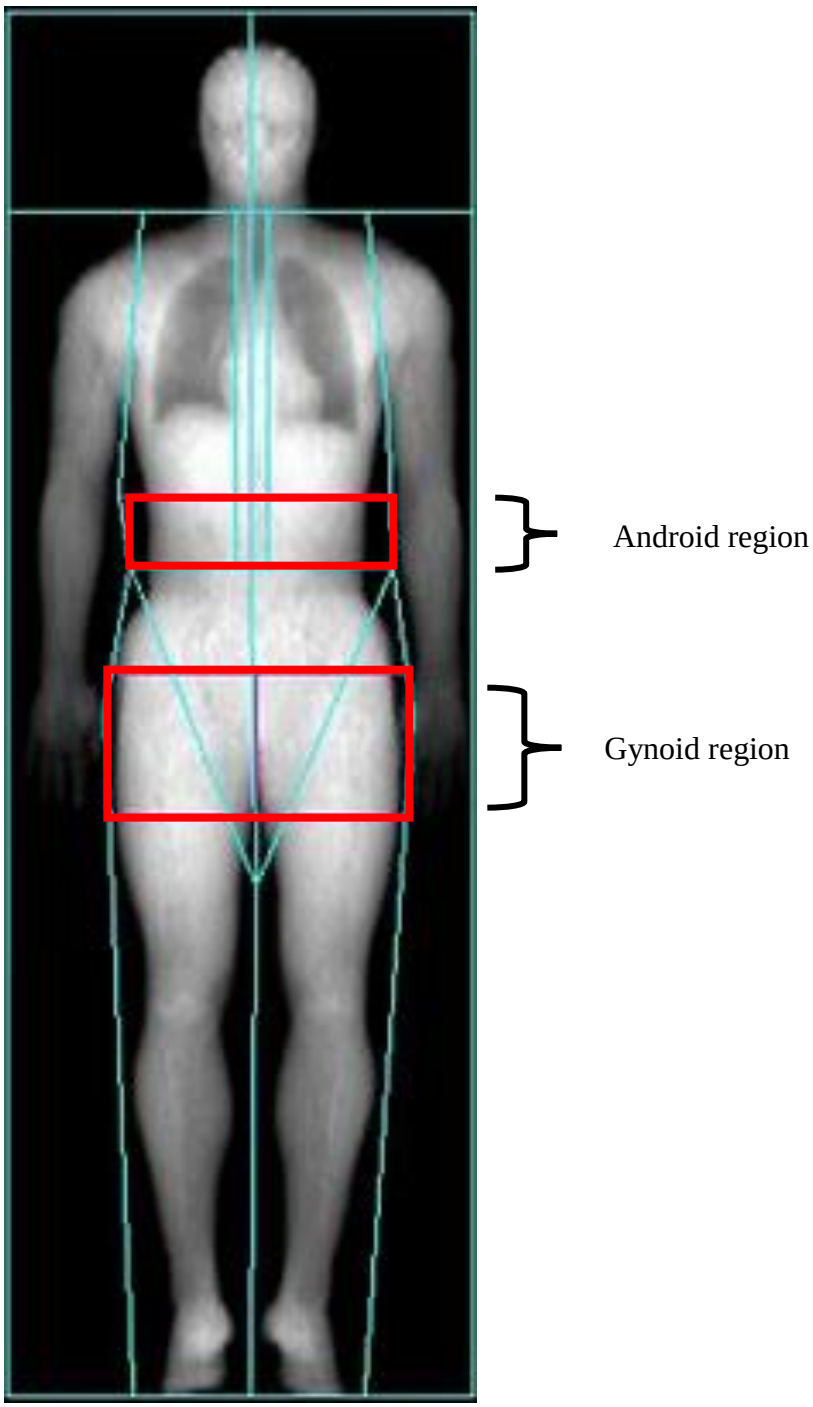

Figure 3. 1: The android and gynoid regions of interest determined by dual energy X-ray absorptiometry

3.3.4 Genotyping

Twelve-hour fasting blood samples were collected and processed at the beginning of the trial, then stored at -80°C until being shipped to the RCFFN for analysis. Genomic DNA was extracted from the buffy coat samples of the first day of the first phase using a Qiagen DNeasy Blood and Tissue Kit according to the manufacturer's instructions (Qiagen Sciences Inc., Toronto, ON, Canada). A Thermo Scientific NanoDrop 2000 micro-volume spectrophotometer was used to assess the concentration and purity of the extracted DNA (Thermo Fisher Scientific Inc., Waltham, MA, USA). TaqMan GTXpress Master Mix with allele-specific probes (Applied Biosystems, Life Technologies Inc., Burlington, ON, Canada) was used for genotyping of the single-nucleotide polymorphisms (SNPs) of interest. Amplification and detection of DNA were conducted with the 7500 Fast Real-Time PCR System (Applied Biosystems, Life Technologies Inc, Burlington, ON, Canada). Data were acquired by software StepOne 2.1 (Applied Biosystems, Life Technologies Inc., Burlington, ON, Canada). Polymorphisms located in lipogenesis/adipogenesis-related genes were selected for their various roles in obesity development, where each SNP chosen was either a functional SNP in lipid-metabolism-related genes, had a higher minor allele frequency (MAF; ≥5), and/or had been previously reported for gene-nutrient interactions. This study assessed possible gene-MUFA interactions in a total of 21 candidate SNPs, summarized in **Table 3.1**, within 17 genes including adiponectin (*ADIPOQ*); adrenoceptor beta-2 (*ADRB2*); apolipoprotein E (*APOE*); endothelial lipase (*LIPG*); fat mass and obesity-associated gene (*FTO*); gastric lipase (*LIPF*); hepatic lipase (*LIPC*); insulin receptor substrate-1 (*IRS-1*); lipase A (*LIPA*); lipoprotein lipase (*LPL*); liver fatty acid binding protein (*FABP1*); perilipin-1 (*PLIN1*); peroxisome proliferator-activated receptor alpha (*PPARα*); *PPARγ*; stearoyl-CoA desaturase

(*SCD*); sterol regulatory element-binding transcription factor-1 (*SREBF1*); transcription factor 7-like 2 (*TCF7L2*).

Table 3.1: Characteristics of the selected single-nucleotide polymorphisms1

Full name of gene	**Gene**	**SNP**	**Region**	**Allele**	**Genotype (n)**			**MAF%**
				Major/minor	MM	Mm	mm	
Adiponectin	*ADIPOQ*	rs266729	Exon/ 5' UTR	C/G	61	30	10	24.8
Adrenoceptor Beta-2	*ADRB2*	rs1042714	Exon	C/G	42	41	18	38.1
Apolipoprotein E	*APOE*	rs429358	Missense	T/C	33	40	28	47.5
		rs7412	Missense	C/T	92	9	0	4.5
		-	-	E3/E4	35	38	28	46.5
Endothelial Lipase	*LIPG*	rs2000813	Missense	C/T	52	43	6	27.2
Fat mass and obesity-associated gene	*FTO*	rs9939609	Intron	T/A	44	39	18	37.1
Gastric lipase	*LIPF*	rs814628	Missense	A/G	72	25	4	16.3
Hepatic lipase	*LIPC*	rs6083	Missense	A/G	37	47	17	40.1
Insulin receptor substrate-1	*IRS-1*	rs2943641	Intergene	C/T	50	42	9	29.7
		rs7578326	Intron	A/G	49	45	7	29.2
Lipase A, lysosomal acid	*LIPA*	rs1051338	Missense	G/T	24	61	16	46.0
Lipoprotein lipase	*LPL*	rs13702	Exon/ 3' UTR	T/C	50	45	6	28.2
		rs3200218	Exon/ 3' UTR	A/G	67	29	5	19.3
Liver fatty acid binding protein	*FABP1*	rs2241883	Missense	T/C	46	45	10	32.2
Perilipin-1	*PLIN1*	rs894160	Intron	C/T	47	43	11	32.2
Peroxisome proliferator-activated receptor alpha	*PPARα*	rs6008259	Exon /3′-UTR	G/A	66	30	5	19.8
Peroxisome proliferator-activated receptor gamma	*PPARγ*	rs1801282	Missense	C/G	78	21	2	12.4
Stearoyl-CoA desaturase (Δ-9-desaturase)	*SCD*	rs2234970	Missense	A/C	43	43	15	36.1
Sterol regulatory element-binding transcription factor-1	*SREBF1*	rs11868035	Intron	G/A	34	54	13	39.6
		rs2297508	Exon/ 3' UTR	G/C	33	52	16	41.6
Transcription factor 7-like 2	*TCF7L2*	rs7903146	Intron	C/T	48	46	7	29.7

[1]MAF: minor allele frequency; MM: major allele homozygous; Mm: heterozygous; mm: minor allele homozygous; SNP: single nucleotide polymorphism.

3.3.5 Statistical analyses

The primary aim of COMIT II trial was to evaluate the effect of MUFA consumption on body composition, mainly visceral adipose tissue. Therefore, the sample size was calculated to detect a 55 gram change using the variance parameter in android fat mass from our previous controlled trial (COMIT I) (1). A total sample size of 140 was required to account for a drop-out rate of 20%.

Statistical analyses were performed using SAS 9.4 (SAS, Cary, NC) based on a per protocol approach. Normality was assessed using the Shapiro–Wilk test and the skewness values. Non-normally distributed variables were log-transformed before analysis. The results are expressed as least-squares means ± SEMs unless otherwise specified and statistical significance was set at $P< 0.05$. Changes in fat mass and body weight represent the difference between endpoint and baseline of each dietary phase. PROC MIXED with repeated-measure procedure was used to assess the effect of the three dietary treatments on changes in body fat and body weight. Treatment, sex, age, and genotype were used as fixed effects, with participants as a repeated factor. Random effects were treatment sequence, clinical site, and participants. Pre-specified potential confounders such as ethnicity, baseline body composition, baseline fasting levels of glucose, homeostatic model of insulin resistance, and cholesterol were investigated in all models. The Hardy-Weinberg equilibrium was assessed with χ^2 test.

For diet-gene interaction analysis, due to the considerably comparable MUFA levels in the two canola treatments compared to the low-MUFA high-SFA treatment, the statistical analysis of MUFA-by-SNP interaction was conducted to compare the combined effect of the two MUFA diets (HOCO+RCO, average) versus the low-MUFA high-SFA diet on changes in body fat and body weight. This decision was also reached based on our inability to detect statistical

differences between HOCO and RCO in body composition in the overall population in our previous trial (COMIT I) (1).

Each individual SNP was assessed separately using the aforementioned statistical model. All SNPs analyzed in the additive model. Dominant and recessive models were analyzed only when the simple effect of heterozygous-by-MUFA showed a significant interaction. Only four *APOE* isoforms (encoded by rs429358 and rs7412) were obtained, and were analyzed and presented as *APOE3* (ε2/ε3 and ε3/ε3) and *APOE4* (ε3/ε4 and ε4/ε4).

3.4 Results

A total of 124 participants (RCFFN= 45, CCARM= 19, INAF= 33, and PSU= 27) completed the trial and all required DXA scans. Three participants were excluded due to high fasting blood glucose (>7mmol/L) and six participants were excluded due to large changes in body weight (>5% endpoint-baseline weight change) at any dietary period (**Figure 3.2**). Therefore, 115 participants (71 women and 44 men) were included in the analysis of the effect of dietary MUFA on changes in body composition. No significant differences were observed in changes in body weight or fat mass following the consumption of any of the three treatments (**Table 3.2)**.

The assessment of gene-by-MUFA interactions included 101 participants (60 women and 41 men; 73% Caucasian), as 14 participants did not consent for genetic analyses. Baseline characteristics are displayed in **Table 3.3**. Genotype-frequencies did not differ from Hardy–Weinberg equilibrium except for the *ADIPOQ* rs266729, *APOE,* and *LIPA* rs10511338. All changes in total and regional fat mass and body weight in response to gene-MUFA interactions are presented in **Table 3.4** and **Table 3.5**. Dietary MUFA was found to interact with common

variants in *LPL*, *FTO*, *PPARα*, *ADIPOQ*, *APOE*, *LIPC*, and *ADRB2* to modify changes in body fatness in an iso-caloric diet (**Figures 3.2-3.7**), as detailed in the following paragraphs.

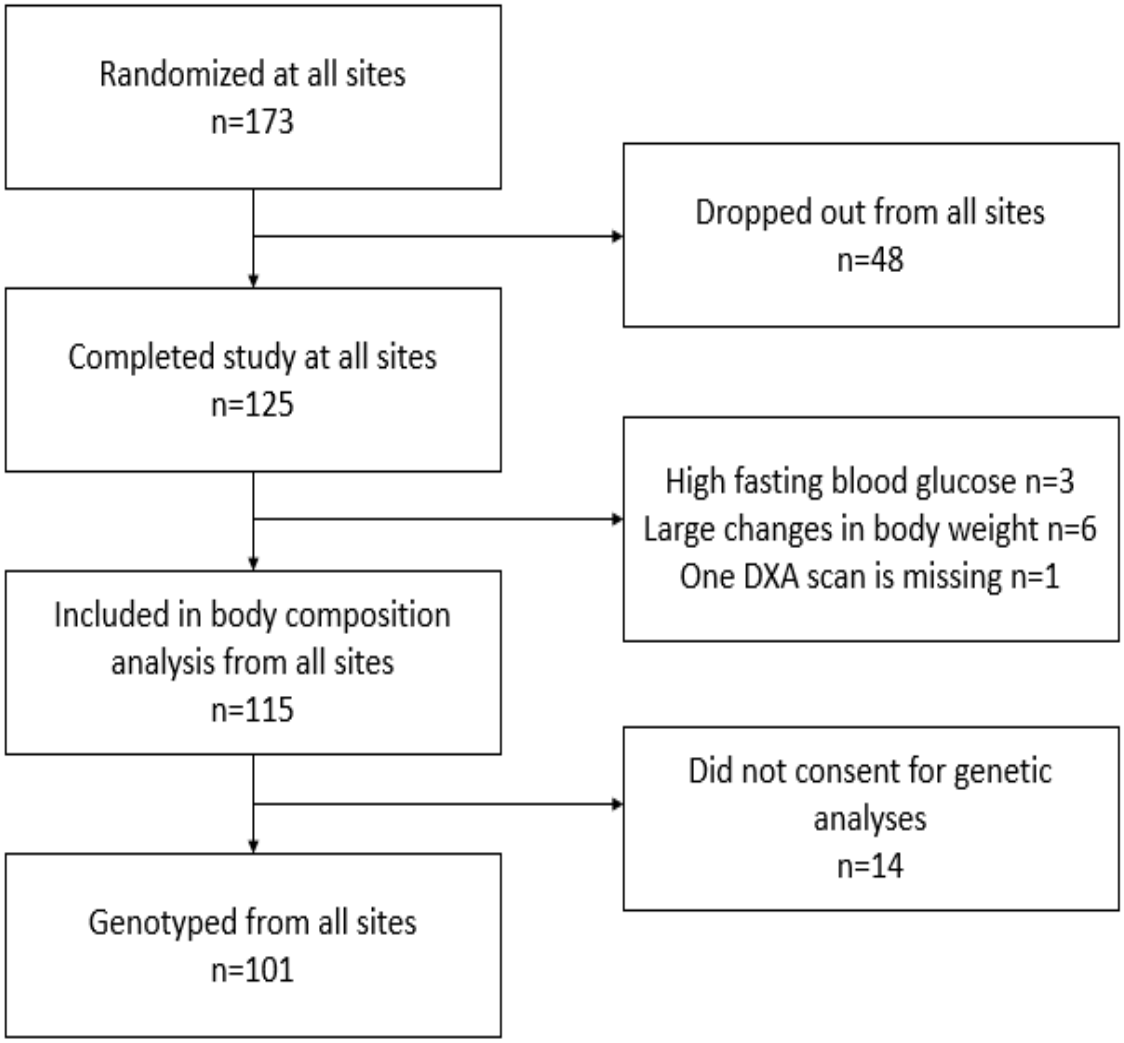

Figure 3. 2: Flow chart of participating adults throughout the study

Table 3.1: Changes in body composition in response to dietary monounsaturated fatty acids[1]

Variable	HOCO	RCO	Low-MUFA high-SFA	*P*[2]
Body weight (g)				0.079
All	-1035.5 ± 236.3	-646.8 ± 236.3	-936.0 ± 236.3	
Female	-990.0 ± 264.0	-753.8 ± 264.0	-836.2 ± 264.0	
Male	-1081.1 ± 306.9	-539.8 ± 306.7	-1035.8 ± 307.0	
Total fat (g)				0.1137
All	-665.2 ± 114.5	-382.8 ± 114.5	-569.1 ± 114.7	
Female	-550.8 ± 142.3	-449.7 ± 142.2	-625.4 ± 142.1	
Male	-779.6 ± 181.2	-315.8 ± 180.9	-512.8 ± 181.5	
Leg fat (g)				0.4153
All	-169.5 ± 56.0	-76.76 ± 55.99	-151.2 ± 56.00	
Female	-149.8 ± 69.5	-139.3 ± 69.44	-123.45 ± 69.44	
Male	-189.2 ± 88.5	-14.17 ± 88.39	-178.9 ± 88.46	
Trunk fat (g)				0.4714
All	-441.1 ± 101.3	-293.3 ± 101.3	-425.9 ± 101.4	
Female	-273.8 ± 125.8	-265.0 ± 125.7	-510.0 ± 125.7	
Male	-608.4 ± 160.2	-321.6 ± 160.0	-341.8 ± 160.2	
Android fat (g)				0.7968
All	-74.38 ± 24.97	-97.25 ± 24.97	-84.15 ± 24.97	
Female	-59.94 ± 30.97	-105.4 ± 30.97	-117.5 ± 30.96	
Male	-88.83 ± 39.43	-89.09 ± 39.42	-50.82 ± 39.40	
Gynoid fat (g)				0.3693
All	-110.8 ± 27.2	-65.49 ± 27.17	-99.57 ± 27.17	
Female	-100.9 ± 33.7	-95.54 ± 33.72	-131.4 ± 33.72	
Male	-120.8 ± 42.9	-35.43 ± 42.94	-67.73 ± 42.95	
VAT mass (g)				0.8853
All	-35.64 ± 19.01	-48.5 6± 19.00	-43.45 ± 19.01	
Female	-18.45 ± 23.56	-34.68 ± 23.58	-58.18 ± 23.56	
Male	-52.83 ± 23.00	-62.44 ± 29.99	-28.73 ± 29.99	
SCAT mass (g)				0.891
All	-39.10 ± 20.14	-50.64 ± 20.14	-37.62 ± 20.14	
Female	-41.22 ± 24.95	-72.23 ± 24.99	-56.57 ± 24.94	
Male	-36.97 ± 31.76	-29.04 ± 31.76	-18.68 ± 31.76	
Total lean (g)				0.6369
All	-359.8 ± 185.8	-247.1 ± 185.7	-371.1 ± 185.7	
Female	-426.2 ± 202.7	-279.8 ± 202.6	-209.9 ± 202.7	
Male	-293.4 ± 230.2	-214.4 ± 230.0	-532.2 ± 229.9	

Table 3.2: Continued

Variable	HOCO	RCO	Low-MUFA high-SFA	*P*
Leg lean (g)				0.6376
All	-85.95 ± 72.47	-9.4 6 ± 72.48	-48.12 ± 72.46	
Female	-101.52 ± 84.33	-69.23 ± 84.31	3.22 ± 84.32	
Male	-70.39 ± 101.92	50.31 ± 101.9	-99.47 ± 101.9	
Trunk lean (g)				0.4505
All	-199.4 ± 134.7	-228.8 ± 134.7	-362.3 ± 134.6	
Female	-191.1 ± 154.2	-179.7 ± 154.1	-249.9 ± 154.2	
Male	-207.8 ± 183.9	-278.0 ± 183.8	-474.6 ± 183.7	
Android lean (g)				0.4262
All	-26.404 ± 21.40	-54.19 ± 21.40	-59.99 ± 21.40	
Female	-48.897 ± 25.91	-58.34 ± 25.91	-54.25 ± 25.91	
Male	-3.9119 ± 32.48	-50.05 ± 32.47	-65.73 ± 32.46	
Gynoid lean (g)				0.8084
All	-7.004 ± 37.82	-32.47 ± 37.82	-15.59 ± 37.83	
Female	-35.26 ± 43.04	-63.40 ± 43.05	21.58 ± 43.03	
Male	21.25 ± 51.02	-1.53 ± 51.02	-52.75 ± 51.01	

[1]All values are least-squares means and SEMs. Changes were calculated as endpoint-baseline values. n=115 participants. HOCO: high-oleic canola oil; RCO: regular canola oil; SCAT: subcutaneous adipose tissue VAT: visceral adipose tissue.

[2]SAS PROC MIXED with repeated measure procedure used to assess the effect of MUFA consumption on body composition changes. Treatment, sex, age, and genotype were used as fixed effects, participants as a repeated factor. Random effects were treatment sequence, clinical site, and participants. $P < 0.05$ was considered significant.

Table 3.2: Characteristics of participants at the baseline of dietary intervention[1]

Characteristic	Total (n=101)	Female (n=60)	Male (n=41)	P^2
Age (years)	43.31 ± 1.29	45.65 ± 1.64	39.88 ± 1.98	0.0271
Ethnicity (n)				-
Caucasian	74	45	29	
African	4	3	1	
Asian	8	4	4	
Hispanic	3	1	2	
Others	12	7	5	
Systolic BP[3] (mmHg)	118.6 ± 1.3	117.8 ± 1.7	119.7 ± 2.0	0.4647
Diastolic BP (mmHg)	78.10 ± 1.08	77.47 ± 1.41	79.00 ± 1.70	0.4915
Total cholesterol (mmol/L)	5.19 ± 0.09	5.21 ± 0.12	5.15 ± 0.14	0.7369
Triglycerides (mmol/L)	1.55 ± 0.07	1.46 ± 0.09	1.67 ± 0.11	0.1523
HDL-cholesterol (mmol/L)	1.35 ± 0.04	1.46 ± 0.04	1.20 ± 0.05	0.0003
LDL-cholesterol (mmol/L)	3.13 ± 0.08	3.09 ± 0.10	3.19 ± 0.12	0.5063
Glucose (mmol/L)	5.22 ± 0.04	5.21 ± 0.06	5.23 ± 0.07	0.8271
Insulin (pmol/L)	98.67 ± 6.10	94.32 ± 7.92	105.03 ± 9.58	0.3908
Waist circumference (cm)	103.8 ± 1.3	100.8 ± 1.6	108.3 ± 1.9	0.003
VAT mass (g)	1334 ± 84	1056 ± 99	1741 ± 120	<.0001
SCAT mass (g)	2213 ± 90	2293 ± 117	2097 ± 141	0.2914
Leg fat mass (g)	12645 ± 430	13790 ± 531	10969 ± 642	0.001
Trunk fat mass (g)	19472 ± 721	18584 ± 930	20772 ± 1124	0.1371
Android fat mass (g)	3548 ± 145	3349 ± 187	3838 ± 226	0.098
Gynoid fat mass (g)	6002 ± 206	6334 ± 264	5516 ± 319	0.0512
Total fat mass (g)	36282 ± 1119	36565 ± 1459	35869 ± 1765	0.7621
Body weight (kg)	89.76 ± 1.88	83.05 ± 2.21	99.59 ± 2.67	<.0001
BMI (kg/m^2)	31.12 ± 0.53	30.99 ± 0.69	31.31 ± 0.84	0.7675

[1]Values are means ± SEMs unless otherwise specified.
[2]SAS PROC MIXED procedure used to assess sex differences, $P<0.05$ was considered significant. SAS PROC MEANS used to determine the mean characteristics of the overall population.
[3]BP: blood pressure; BMI: body mass index; HDL: high-density lipoprotein; LDL: low-density lipoprotein; SCAT: subcutaneous adipose tissue; VAT: visceral adipose tissue

Table 3.3: Changes in trunk, leg, and total fat mass, and body weight following dietary treatments by the selected polymorphisms[1]

Gene, SNP, allele (n)	Change trunk fat, g			Change leg fat, g			Change total fat, g			Change weight, g		
	High MUFA	Low MUFA	*P*[2]	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*
ADIPOQ rs266729			0.418			0.92			0.277			0.333
C/C (61)	-363.2 ± 111.4	-543.1 ± 139.8		-190.9 ± 54.2	-240.9 ± 72.7		-571.9 ± 123.2	-793.3 ± 151.2		-876.6 ± 233.1	-1134.6 ± 262.6	
C/G (30)	-409.7 ± 153.1	-698.3 ± 194.3		-64.7 ± 77.8	-59.2 ± 103.7		-541.0 ± 177.1	-711.5 ± 215.6		-1011.8 ± 299.9	-740.5 ± 344.9	
G/G (10)	-381.6 ± 258.3	-119.0 ± 328.5		-60.2 ± 135.0	-61.8 ± 178.8		-512.8 ± 308.6	-88.8 ± 370.5		-898.8 ± 471.3	-1091.4 ± 552.8	
ADRB2 rs1042714			0.571			0.641			0.07			0.502
C/C (42)	-335.0 ± 131.6	-505.4 ± 166.3		-105.0 ± 65.8	-175.9 ± 87.9		-461.6 ± 147.9	-691.5 ± 180.9		-869.4 ± 262.1	-1110.1 ± 299.9	
C/G (41)	-366.7 ± 133.3	-431.5 ± 167.8		-190.4 ± 67.0	-145.3 ± 88.8		-654.7 ± 151.1	-522.4 ± 182.6		-899.8 ± 263.1	-767.6 ± 300.4	
G/G (18)	-497.1 ± 193.3	-899.9 ± 248.0		-110.0 ± 100.4	-203.7 ± 134.0		-547.7 ± 225.7	-1136.7 ± 275.7		-1076.3 ± 360.3	-1354.3 ± 423.3	
APOE[3]			0.011			0.989			0.009			0.36
E3 (35)	-517.0 ± 140.0	-295.7 ± 176.8		-112.4 ± 72.0	-139.9 ± 95.7		-668.4 ± 162.3	-402.0 ± 195.9		-1034.0 ± 303.3	-925.8 ± 341.7	
E4 (66)	-302.9 ± 106.6	-677.7 ± 133.2		-155.2 ± 52.7	-184.6 ± 70.3		-497.8 ± 118.6	-862.2 ± 144.7		-868.3 ± 238.0	-1070.2 ± 265.1	
FABP1 rs2241883			0.488			0.684			0.847			0.579
C /C (10)	-371.5 ± 254.5	-931.3 ± 331.4		-170.8 ± 133.3	-172.5 ± 179.7		-625.0 ± 303.0	-957.8 ± 376.1		-1102.1 ± 469.3	-1682.7 ± 558.7	
T/C (45)	-407.9 ± 126.2	-502.9 ± 159.7		-41.8 ± 63.1	-131.6 ± 84.5		-511.5 ± 144.0	-615.7 ± 176.0		-902.5 ± 259.0	-901.3 ± 294.5	
T/T (46)	-339.9 ± 126.0	-496.0 ± 158.9		-230.1 ± 63.0	-202.7 ± 84.0		-572.6 ± 144.1	-722.3 ± 175.4		-889.7 ± 259.6	-970.3 ± 294.2	

Table 3.4: Continued

Gene, SNP, allele (n)	Change trunk fat, g			Change leg fat, g			Change total fat, g			Change weight, g		
	High MUFA	Low MUFA	P^2	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*
FTO rs9939609			0.854			0.837			0.831			0.053
A/A (18)	-366.2 ± 192.0	-434.5 ± 246.1		26.0 ± 100.1	-82.9 ± 133.3		-270.8 ± 225.8	-441.3 ± 274.9		-885.3 ± 349.9	-1192.2 ± 411.7	
A/T (39)	-292.2 ± 130.6	-532.2 ± 168.4		-158.5 ± 67.1	-182.1 ± 90.4		-516.5 ± 150.5	-738.5 ± 186.7		-606.3 ± 250.2	-1084.9 ± 291.8	
T/T (44)	-454.3 ± 126.2	-598.4 ± 160.2		-191.6 ± 64.7	-192.3 ± 85.9		-702.9 ± 146.1	-772.0 ± 177.6		-1188.8 ± 240.5	-842.1 ± 277.8	
IRS rs2943641			0.241			0.979			0.276			0.247
C/C (50)	-384.21 ± 122.72	-485.07 ± 153.0		-96.8 ± 61.8	-137.6 ± 81.3		-496.1 ± 139.9	-593.5 ± 168.0		-781.3 ± 258.5	-775.9 ± 289.6	
C/T (42)	-333.16 ± 128.33	-682.99 ± 163.8		-183.7 ± 65.1	-197.0 ± 87.5		-580.3 ± 146.0	-892.8 ± 180.6		-998.9 ± 268.5	-1360.2 ± 305.9	
T/T (9)	-533.31 ± 270.66	-231.56 ± 347.1		-160.3 ± 141.7	-198.2 ± 189.0		-734.0 ± 319.3	-394.4 ± 389.0		-1337.5 ± 489.9	-788.7 ± 579.3	
IRS rs7578326			0.407			0.915			0.58			0.372
A/A (49)	-353.2 ± 124.2	-590.8 ± 155.3		-146.2 ± 62.7	-155.6 ± 82.5		-540.3 ± 141.8	-713.2 ± 171.1		-889.6 ± 252.3	-945.6 ± 285.6	
A/G (45)	-367.1 ± 125.0	-548.9 ± 159.0		-135.3 ± 63.1	-170.7 ± 84.7		-540.4 ± 141.8	-732.1 ± 174.9		-876.9 ± 252.3	-1133.0 ± 288.9	
G/G (7)	-581.6 ± 307.0	-204.8 ± 392.9		-130.2 ± 161.6	-248.0 ± 214.7		-720.9 ± 364.9	-425.3 ± 442.2		-1368.6 ± 547.8	-711.9 ± 649.8	
LIPA rs1051338			0.5			0.681			0.255			0.608
G/G (24)	-311.5 ± 162.7	-278.6 ± 212.5		-205.6 ± 85.4	-227.0 ± 115.7		-582.1 ± 191.9	-420.8 ± 239.6		-1007.2 ± 334.8	-820.4 ± 387.9	
G/T (61)	-322.0 ± 109.1	-595.6 ± 137.2		-138.6 ± 55.2	-136.1 ± 73.1		-494.8 ± 125.4	-784.5 ± 151.1		-867.2 ± 240.6	-1046.1 ± 268.5	
T/T (16)	-670.7 ± 200.2	-763.3 ± 259.9		-41.2 ± 106.1	-197.6 ± 142.3		-699.2 ± 239.3	-822.3 ± 294.7		-986.0 ± 398.0	-1195.5 ± 463.4	

Table 3.4: Continued

Gene, SNP, allele (n)	Change trunk fat, g			Change leg fat, g			Change total fat, g			Change weight, g		
	High MUFA	Low MUFA	*P*[2]	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*
LIPC rs6083			0.335			0.374			0.048			0.93
A/A (37)	-490.0 ± 139.2	-467.7 ± 176.4		-183.3 ± 69.5	-99.5 ± 93.3		-705.3 ± 157.3	-526.7 ± 193.0		-995.2 ± 273.1	-1016.0 ± 314.7	
A/G (47)	-385.8 ± 128.4	-611.5 ± 159.4		-136.9 ± 62.9	-210.1 ± 83.0		-576.0 ± 143.7	-828.1 ± 172.1		-919.5 ± 252.5	-1075.6 ± 286.5	
G/G (17)	-98.4 ± 197.0	-534.9 ± 254.3		-53.9 ± 102.3	-205.1 ± 137.5		-155.4 ± 229.4	-744.1 ± 283.4		-742.9 ± 366.8	-839.8 ± 433.9	
LIPF rs814628			0.235			0.824			0.165			0.408
A/A (72)	-415.5 ± 107.0	-492.8 ± 131.9		-162.0 ± 50.8	-184.0 ± 67.5		-600.8 ± 114.5	-662.9 ± 139.5		-940.3 ± 215.3	-1094.6 ± 242.3	
A/G (25)	-295.9 ± 167.1	-790.6 ± 211.8		-81.1 ± 85.0	-155.4 ± 113.4		-440.0 ± 191.6	-937.9 ± 233.0		-999.0 ± 308.6	-792.0 ± 360.6	
G/G (4)	-146.4 ± 393.1	13.6 ± 515.3		-126.4 ± 210.1	7.9 ± 283.7		-355.9 ± 470.6	34.4 ± 585		0.4 ± 706.3	-821.5 ± 852.4	
LIPG rs2000813			0.33			0.934			0.546			0.936
C/C (52)	-359.1 ± 117.9	-518.6 ± 148.9		-157.3 ± 58.5	-202.5 ± 78.4		-530.5 ± 132.4	-725.3 ± 162.5		-920.0 ± 244.1	-980.6 ± 277.0	
C/T (43)	-360.5 ± 129.1	-632.3 ± 163.0		-88.0 ± 64.6	-89.8 ± 86.3		-497.8 ± 146.4	-660.8 ± 178.8		-871.1 ± 260.4	-1028.9 ± 297.1	
T/T (6)	-617.4 ± 328.8	-158.1 ± 422.9		-367.5 ± 172.3	-441.3 230.4		-1144.0 ± 390.4	-790.6 ± 476.8		-1238.9 ± 595.9	-1195.0 ± 708.6	
LPL rs13702			0.051			0.61			0.074			0.07
C/C (6)	-1185.1 ± 318.6	-269.0 ± 413.3		-262.3 ± 172.5	-459.8 ± 230.4		-1495.0 ± 385.1	-599.9 ± 471.0		-2124.3 ± 584.3	-839.9 ± 696.2	
C/T (45)	-449.6 ± 119.7	-704.7 ± 153.7		-107.5 ± 63.2	-73.0 ± 84.4		-566.7 ± 141.2	-759.8 ± 172.7		-895.1 ± 261.0	-1218.0 ± 295.5	
T/T (50)	-204.2 ± 114.4	-422.3 ± 146.8		-154.7 ± 60.2	-219.5 ± 80.4		-423.8 ± 134.3	-653.0 ± 164.8		-806.8 ± 250.9	-852.5 ± 283.5	

Table 3.4: Continued

Gene, SNP, allele (n)	Change trunk fat, g			Change leg fat, g			Change total fat, g			Change weight, g		
	High MUFA	Low MUFA	P^2	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*
LPL rs3200218			0.775			0.837			0.81			0.613
A/A (67)	-394.5 ± 110.5	-616.1 ± 136.5		-157.3 ± 52.9	-205.9 ± 70.1		-569.6 ± 119.7	-764.9 ± 145.4		-1043.5 ± 220.3	-1071.2 ± 248.4	
A/G (29)	-304.5 ± 154.4	-349.2 ± 197.1		-121.9 ± 78.5	-92.2 ± 105.3		-491.3 ± 176.6	-524.9 ± 217.7		-609.8 ± 291.1	-925.4 ± 340.2	
G/G (5)	-571.8 ± 357.0	-788.7 ± 463.0		-29.4 ± 189.2	-126.6 ± 253.7		-692.7 ± 426.1	-913.4 ± 524.3		-1041.1 ± 635.3	-730.6 ± 762.9	
PLIN rs894160			0.377			0.658			0.843			0.41
C/C (47)	-442.6 ± 128.5	-475.0 ± 159.1		-112.8 ± 64.7	-204.4 ± 84.4		-585.2 ± 147.4	-688.2 ± 175.4		-942.0 ± 265.9	-817.1 ± 298.0	
C/T (43)	-314.3 ± 128.0	-531.8 ± 162.7		-174.6 ± 64.5	-157.9 ± 86.5		-536.3 ± 145.1	-685.1 ± 179.0		-961.3 ± 264.2	-1292.4 ± 300.8	
T/T (11)	-360.2 ± 238.5	-891.8 ± 314.3		-113.3 ± 125.7	-58.5 ± 171.3		-500.2 ± 281.5	-822.8 ± 356.0		-705.7 ± 443.6	-758.3 ± 530.7	
PPARα rs6008259			0.484			0.064			0.253			0.544
A/A (5)	-590.0 ± 370.3	-857.8 ± 462.5		-368.4 ± 193.4	281.3 ± 251.6		-1017.2 ± 445.9	-548.7 ± 519.1		-613.9 657.7	-1304.2 ± 767.4	
A/G (30)	-362.7 ± 158.9	-731.5 ± 199.1		-104.4 ± 79.6	-189.8 ± 104.8		-499.3 ± 181.3	-887.9 ± 219.1		-977.4 300.9	-1230.7 ± 346.2	
G/G (66)	-367.5 ± 111.2	-439.8 ± 137.5		-140.4 ± 51.8	-191.5 ± 69.3		-547.1 ± 117.5	-628.1 ± 144.3		-912.4 222.6	-897.3 ± 251.1	
PPARγ rs1801282			0.656			0.614			0.646			0.085
C/C (78)	-383.1 ± 97.1	-554.8 ± 122.7		-155.2 ± 48.3	-159.9 ± 64.6		-571.9 ± 108.7	-678.6 ± 134.0		-886.4 ± 203.4	-1097.8 ± 229.7	
C/G (21)	-404.8 ± 182.0	-503.1 ± 228.7		-87.6 ± 94.9	-231.7 ± 124.3		-528.1 ± 216.1	-801.0 ± 256.2		-982.9 ± 336.6	-479.6 ± 389.6	
G/G (2)	238.0 ± 586.9	-661.7 ± 731.7		-93.9 ± 310.2	101.8 ± 402.2		63.2 ± 712.7	-734.7 ± 827.5		-959.1 ± 1022.3	-2623.9 ± 1195.2	

Table 3.4: Continued

Gene, SNP, allele (n)	Change trunk fat, g			Change leg fat, g			Change total fat, g			Change weight, g		
	High MUFA	Low MUFA	*P*[2]	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*
SCD rs2234970			0.145			0.348			0.47			0.908
A/A (43)	-383.0 ± 129.0	-508.8 ± 163.1		-155.9 ± 64.2	-282.7 ± 85.8		-577.1 ± 145.3	-803.2 ± 178.2		-1067.5 ± 276.1	-1139.5 ± 311.2	
A/C (43)	-268.9 ± 128.9	-636.7 ± 163.1		-79.5 ± 64.3	-5.7 ± 85.9		-364.7 ± 145.3	-541.7 ± 178.6		-686.8 ± 275.3	-744.2 ± 310.8	
C/C (15)	-672.6 ± 212.3	-378.3 ± 268.8		-268.3 ± 109.8	-304.3 ± 145.0		-1042.7 ± 248.4	-842.6 ± 299.3		-1258.7 ± 405.3	-1525.0 ± 469.8	
SREBF1 rs11868035			0.396			0.794			0.918			0.92
A/A (13)	-246.6 ± 116.3	-187.2 ± 156.7		-244.0 ± 220.7	-481.9 ± 288.0		-670.9 ± 260.8	-704.0 ± 324.2		-1066.0 ± 407.6	-1330.0 ± 485.9	
A/G (54)	-178.0 ± 58.0	-151.5 ± 77.3		-428.7 ± 115.0	-647.5 ± 145.8		-625.4 ± 130.7	-804.7 ± 160.1		-965.4 ± 235.7	-1036.5 ± 268.4	
G/G (34)	-36.1 ± 73.1	-187.6 ± 97.2		-341.8 ± 142.3	-402.5 ± 181.4		-385.9 ± 164.7	-533.2 ± 201.0		-763.9 ± 282.8	-830.6 ± 325.0	
SREBF1 rs2297508			0.639			0.355			0.928			0.8
C/C (16)	-192.1 ± 199.6	-577.0 ± 259.7		-257.4 ± 105.3	-130.5 ± 141.3		-615.8 ± 236.0	-684.9 ± 292.4		-930.1 ± 376.1	-1269.4 ± 444.7	
C/G (52)	-453.0 ± 117.4	-621.8 ± 148.7		-165.3 ± 59.5	-169.2 ± 79.0		-625.9 ± 134.0	-814.0 ± 163.8		-986.9 ± 241.5	-1037.4 ± 274.6	
G/G (33)	-346.4 ± 143.8	-406.3 ± 183.6		-40.6 ± 74.2	-185.3 ± 98.6		-404.0 ± 166.9	-530.9 ± 204.0		-792.6 ± 286.9	-833.7 ± 329.8	

Table 3.4: Continued

Gene, SNP, allele (n)	Change trunk fat, g			Change leg fat, g			Change total fat, g			Change weight, g		
	High MUFA	Low MUFA	*P*[2]	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*
TCF7L2 rs7903146			0.855			0.931			0.966			0.82
C/C (48)	-261.5 ± 127.1	-448.0 ± 158.5		-155.2 ± 61.4	-157.0 ± 81.9		-508.9 ± 140.9	-628.7 ± 171.2		-819.6 ± 264.3	-841.4 ± 296.9	
C/T (46)	-509.1 ± 130.6	-694.2 ± 162.5		-157.8 ± 62.9	-207.4 ± 83.8		-661.6 ± 144.7	-822.8 ± 175.5		-1101.7 ± 269.5	-1220.1 ± 302.9	
T/T (7)	-311.6 ± 296.2	-257.5 ± 393.6		71.6 ± 156.3	15.0 ± 214.2		-180.9 ± 350.0	-407.5 ± 446.2		-589.9 ± 545.4	-1002.0 ± 660.1	

[1]All values are least-squares means and SEMs. Changes were calculated as endpoint-baseline values. n=101 participants. *ADIPOQ*: adiponectin; *ADRB2*: adrenoceptor Beta-2; *APOE*: apolipoprotein E; *FABP1*: liver fatty acid binding protein; *FTO*: fat mass and obesity-associated gene; *IRS1*: Insulin receptor substrate-1; *LIPA*: lipase A, lysosomal acid; *LIPC*: hepatic lipase; *LIPF*: gastric lipase; *LIPG*: endothelial Lipase; low-MUFA: low-MUFA high-Saturated fatty acid diet; *LPL*: lipoprotein lipase; MUFA: monounsaturated fatty acids; *PLIN*: perilipin-1; *PPARα*: peroxisome proliferator-activated receptor alpha; *PPARγ*: peroxisome proliferator-activated receptor gamma; *SCD*: stearoyl-CoA desaturase; SNP: single nucleotide polymorphism; *SREBF1*: sterol regulatory element-binding transcription factor 1; *TCF7L2*: transcription factor 7-like 2.
[2]SAS PROC MIXED with repeated measure procedure used to assess the effect of gene-MUFA interactions on fat mass changes, using participants as a repeated factor. Treatment, sex, age, and genotype were used as fixed effects. *P* interaction <0.05 was considered significant. [3]*APOE3* is the sum of ε2/ε3 and ε3/ε3 and *APOE4* is the sum of ε3/ε4 and ε4/ε4.

Table 3.4: Changes in android, gynoid, visceral, and subcutaneous fat mass following dietary treatments by the selected polymorphisms[1]

Gene, SNP, allele (n)	Change android fat, g			Change gynoid fat, g			Change VAT, g			Change SCAT, g		
	High MUFA	Low MUFA	*P*[2]	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*
ADIPOQ rs266729			0.084			0.638			0.306			0.062
C/C (61)	-74.1 ± 24.5	-107.3 ± 32.6		-106.3 ± 28.5	-148.6 ± 36.2		-46.1 ± 19.2	-38.6 ± 26.1		-28.9 ± 17.6	-66.3 ± 25.6	
C/G (30)	-97 ± 35.1	-178.4 ± 46.5		-99.3 ± 40.9	-107.5 ± 51.7		-30.2 ± 27.3	-102.5 ± 37.2		-68.5 ± 25.1	-71.2 ± 36.5	
G/G (10)	-88.8 ± 60.4	65.1 ± 80.2		-80.9 ± 70.6	-31.9 ± 89.0		-28.6 ± 46.5	-13.5 ± 64.2		-63.9 ± 42.8	100.9 ± 63.0	
ADRB2 rs1042714			0.405			0.297			0.938			0.249
C/C (42)	-76.9 ± 29.6	-130.4 ± 39.4		-57.8 ± 34.0	-135.3 ± 43.3		-50.9 ± 23.0	-56.6 ± 31.5		-28 ± 21.1	-69 ± 30.9	
C/G (41)	-81.2 ± 30.0	-66 ± 39.9		-122.3 ± 34.6	-106.3 ± 43.7		-32.7 ± 23.1	-54 ± 31.8		-48.8 ± 21.2	-4.3 ± 31.2	
G/G (18)	-91.3 ± 45.2	-171.9 ± 60.1		-156.1 ± 51.9	-139.7 ± 66.0		-27.3 ± 35.1	-53.6 ± 48.0		-69.7 ± 32.2	-112.3 ± 47.1	
APOE[3]			0.205			0.823			0.227			0.833
E3 (35)	-88.8 ± 32.3	-69.4 ± 43.0		-80.4 ± 37.3	-94.5 ± 47.3		-52 ± 24.8	-28.2 ± 34.2		-37.3 ± 23.1	-36.5 ± 34.0	
E4 (66)	-77.1 ± 23.8	-134.7 ± 31.5		-112.6 ± 27.4	-140.6 ± 34.8		-32.3 ± 18.4	-69.5 ± 25.1		-47.5 ± 17.1	-58 ± 24.8	
FABP1 rs2241883			0.491			0.474			0.823			0.662
C /C (10)	-79.8 ± 61.2	-214.1 ± 81.3		-126.4 ± 70.5	-61.8 ± 89.6		-92.7 ± 47.0	-153.9 ± 64.1		16 ± 44.0	-59.8 ± 63.5	
T/C (45)	-79.3 ± 28.8	-99 ± 38.2		-86.6 ± 33.6	-96.8 ± 42.3		-52.4 ± 21.9	-65.3 ± 30.2		-29.7 ± 20.5	-25 ± 29.9	
T/T (46)	-82.1 ± 28.7	-100.3 ± 38.0		-110.9 ± 33.5	-166 ± 42.1		-11.9 ± 21.7	-20.3 ± 29.9		-72.2 ± 20.3	-75.3 ± 29.6	
FTO rs9939609			0.364			0.526			0.938			0.084
A/A (18)	-50.1 ± 44.9	-75.6 ± 59.8		-35.5 ± 51.9	-59.2 ± 65.9		-12 ± 34.8	-34.9 ± 48.0		-39.4 ± 31.7	-36.8 ± 46.8	
A/T (39)	-45.4 ± 30.3	-123.6 ± 40.6		-97.7 ± 35.0	-160.4 ± 44.7		-45.2 ± 23.8	-50 ± 32.6		2.2 ± 21.7	-72.7 ± 31.8	
T/T (44)	-127.6 ± 29.1	-114.7 ± 38.6		-130.5 ± 33.7	-118.6 ± 42.5		-45.4 ± 22.6	-67.9 ± 30.9		-87.4 ± 20.5	-37.7 ± 30.1	

Table 3.5: Continued

Gene, SNP, allele (n)	Change android fat, g			Change gynoid fat, g			Change VAT, g			Change SCAT, g		
	High MUFA	Low MUFA	P^2	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*
IRS rs2943641			0.272			0.377			0.392			0.758
C/C (50)	-77.8 ± 27.8	-102.9 ± 36.5		-82.7 ± 32.8	-102.9 ± 40.8		-34.5 ± 21.3	-60.8 ± 29.0		-46.1 ± 19.8	-36.3 ± 28.8	
C/T (42)	-74 ± 29.5	-142.5 ± 39.3		-94 ± 34.5	-146.5 ± 43.7		-40.6 ± 23.0	-66 ± 31.3		-36.8 ± 21.4	-66.2 ± 31.1	
T/T (9)	-127.7 ± 63.9	-25.9 ± 84.9		-241 ± 73.7	-140.9 ± 93.4		-58.5 ± 49.2	31 ± 67.6		-61.1 ± 45.8	-56.8 ± 67.1	
IRS rs7578326			0.372			0.714			0.344			0.816
A/A (49)	-67.2 ± 28.2	-131.3 ± 37.0		-83.3 ± 32.9	-115.1 ± 41.2		-27.7 ± 21.6	-66.5 ± 29.4		-39.7 ± 20.1	-61 ± 29.1	
A/G (45)	-87.8 ± 28.5	-101.5 ± 38.0		-103.4 ± 33.2	-131.1 ± 42.1		-46.8 ± 22.1	-54.9 ± 30.2		-46.9 ± 20.6	-35.4 ± 30.0	
G/G (7)	-131.9 ± 72.6	-42.7 ± 96.4		-208.4 ± 83.8	-142 ± 106.1		-69.8 ± 55.6	30.8 ± 76.6		-54.6 ± 51.7	-74.7 ± 76.1	
LIPA rs1051338			0.552			0.145			0.05			0.617
G/G (24)	-52.6 ± 38.7	-57 ± 51.9		-148.2 ± 44.5	-100.9 ± 56.9		-27.2 ± 30.3	-13.1 ± 41.0		-34.5 ± 28.4	-37.1 ± 41.1	
G/T (61)	-79.8 ± 24.8	-135.4 ± 32.8		-63.5 ± 28.6	-134.6 ± 36.0		-25 ± 18.8	-81.6 ± 25.8		-54.5 ± 17.7	-48.3 ± 25.9	
T/T (16)	-132.1 ± 47.9	-109.1 ± 63.9		-169.4 ± 55.2	-122 ± 70.1		-118.6 ± 37.1	-16 ± 50.4		-17 ± 34.8	-81.1 ± 50.5	
LIPC rs6083			0.239			0.543			0.251			0.717
A/A (37)	-110.3 ± 31.4	-95.7 ± 41.8		-144.9 ± 36.1	-125.1 ± 46.1		-43.9 ± 24.8	-35.3 ± 33.6		-71.4 ± 22.5	-49.6 ± 33.0	
A/G (47)	-80.2 ± 28.2	-110.8 ± 37.3		-94.5 ± 32.6	-142.8 ± 41.1		-62.3 ± 22.1	-66.2 ± 30.0		-18 ± 19.8	-40.9 ± 29.3	
G/G (17)	-19.7 ± 46.3	-148.7 ± 61.7		-22.6 ± 53.0	-71 ± 67.8		34.3 ± 36.2	-70.8 ± 49.2		-57.6 ± 33.3	-74.1 ± 48.7	

Table 3.5: Continued

Gene, SNP, allele (n)	Change android fat, g			Change gynoid fat, g			Change VAT, g			Change SCAT, g		
	High MUFA	Low MUFA	P^2	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*
LIPF rs814628			0.303			0.174			0.752			0.440
A/A (72)	-86.7 ± 22.8	-92.6 ± 30.2		-118.5 ± 27.2	-130.1 ± 33.9		-37.6 ± 17.6	-49.3 ± 24.1		-50.4 ± 16.4	-37.6 ± 23.8	
A/G (25)	-82.9 ± 38.0	-191.6 ± 50.7		-56.7 ± 44.5	-149 ± 56.2		-50.1 ± 29.3	-88 ± 40.5		-35.1 ± 27.3	-98 ± 40.1	
G/G (4)	31.9 ± 94.4	40.2 ± 126.6		-65.1 ± 109.3	123.6 ± 139.5		-0.7 ± 74.4	55 ± 101.4		19.8 ± 69.1	10.3 ± 100.3	
LIPG rs2000813			0.702			0.656			0.949			0.831
C/C (52)	-91.1 ± 26.7	-115.5 ± 35.5		-97.9 ± 30.7	-146 ± 39.0		-37.6 ± 20.6	-54.1 28.3		-55.7 ± 19.1	-59.2 ± 27.9	
C/T (43)	-68.6 ± 29.4	-118.7 ± 39.1		-93.7 ± 33.9	-95 ± 42.9		-37 ± 22.7	-56.1 ± 31.1		-34 ± 21.0	-51.7 ± 30.7	
T/T (6)	-81.7 ± 78.4	-28.5 ± 104.3		-186.3 ± 90.3	-148.7 ± 114.6		-70.4 ± 60.5	-55.6 ± 83.2		-10.8 ± 55.9	37.8 ± 82.1	
LPL rs13702			0.040			0.124			0.027			0.859
C/C (6)	-267.3 ± 76.4	-21.7 ± 102.2		-152.8 ± 89.8	-246.5 ± 113.8		-216.2 ± 58.6	17.2 ± 81.1		-57 ± 56.0	-29.8 ± 82.2	
C/T (45)	-93.4 ± 28.0	-165.8 ± 37.4		-131.3 ± 32.9	-86.9 ± 41.7		-46.2 ± 21.5	-92 ± 29.7		-46.9 ± 20.5	-67.7 ± 30.1	
T/T (50)	-46.8 ± 26.7	-72.6 ± 35.6		-67.8 ± 31.4	-142.8 ± 39.7		-10.8 ± 20.6	-30.2 ± 28.3		-39.5 ± 19.6	-37.3 ± 28.6	
LPL rs3200218			0.64			0.849			0.398			0.04
A/A (67)	-89.6 ± 23.8	-103.7 ± 31.5		-98.1 ± 27.6	-115.7 ± 34.8		-44 ± 18.1	-83.1 ± 24.9		-49.3 ± 16.9	-12.9 ± 24.5	
A/G (29)	-51 ± 35.5	-123.7 ± 47.3		-114.2 ± 40.9	-159 ± 52.1		-19.4 ± 27.2	13.4 ± 37.4		-29.3 ± 25.4	-136.2 ± 37.0	
G/G (5)	-150 ± 85.5	-144.6 ± 114.0		-61.4 ± 98.6	-32.2 ± 125.5		-105.2 ± 65.4	-94 ± 90.2		-43.9 ± 61.0	-53.4 ± 89.0	

Table 3.5: Continued

Gene, SNP, allele (n)	Change android fat, g			Change gynoid fat, g			Change VAT, g			Change SCAT, g		
	High MUFA	Low MUFA	P^2	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*
PLIN rs894160			0.259			0.962			0.685			0.148
C/C (47)	-108.8 ± 29.0	-89.3 ± 37.9		-75 ± 33.5	-90.9 ± 41.8		-54.4 ± 22.1	-69.1 ± 30.1		-56 ± 20.4	-11.1 ± 29.6	
C/T (43)	-58 ± 29.1	-122.9 ± 38.8		-125.8 ± 33.5	-158.6 ± 42.7		-27.6 ± 22.6	-30.4 ± 31.0		-30.1 ± 20.9	-89.7 ± 30.6	
T/T (11)	-59.8 ± 57.4	-164.1 ± 76.7		-107.4 ± 65.6	-124.1 ± 84.5		-23.3 ± 45.3	-98.7 ± 61.3		-40.8 ± 42.0	-64.1 ± 60.5	
PPARα rs6008259			0.56			0.442			0.077			0.196
A/A (5)	-138.6 ± 85.9	-281.9 ± 113.7		-155.3 ± 100.0	-47 ± 125.4		-39 ± 64.5	-61.8 ± 90.2		-99.6 ± 59.1	-224.8 ± 89.1	
A/G (30)	-65.3 ± 35.9	-117.5 ± 47.3		-88.7 ± 41.8	-81.9 ± 52.4		-11.1 ± 27.8	-108.4 ± 37.5		-56 ± 25.4	-1.2 ± 36.8	
G/G (66)	-83.7 ± 23.4	-95.8 ± 31.3		-101.8 ± 27.1	-148.3 ± 34.5		-52.3 ± 18.2	-29.8 ± 24.9		-34.1 ± 16.7	-59.7 ± 24.5	
PPARγ rs1801282			0.811			0.738			0.201			0.077
C/C (78)	-81.3 ± 21.9	-119.3 ± 29.1		-116.5 ± 25.1	-130.1 ± 31.9		-45.9 ± 17.0	-37.7 ± 23.0		-36.6 ± 15.7	-74.4 ± 22.7	
C/G (21)	-88.9 ± 42.5	-87.1 ± 56.0		-50.1 ± 48.9	-117.2 ± 61.4		-23 ± 32.0	-116 ± 44.4		-71.8 ± 29.6	30.3 ± 43.7	
G/G (2)	-0.2 ± 138.0	-101 ± 181.3		-34 ± 159.1	5.7 ± 198.9		12 ± 102.8	-84.1 ± 143.6		-12.3 ± 95.0	16.3 ± 141.6	
SCD rs2234970			0.264			0.674			0.597			0.267
A/A (43)	-84.3 ± 29.2	-74.9 ± 38.9		-120.7 ± 34.1	-153 ± 43.2		-28.1 ± 22.6	-45.1 ± 31.0		-63.4 ± 21.0	-22.9 ± 30.7	
A/C (43)	-60.4 ± 29.3	-145.7 ± 38.9		-69.8 ± 34.2	-66.2 ± 43.2		-32.2 ± 22.7	-67 ± 31.0		-26 ± 21.1	-73.4 ± 30.7	
C/C (15)	-131.7 ± 49.6	-119.1 ± 65.7		-137.9 ± 57.4	-210.3 ± 72.3		-90.5 ± 37.8	-52 ± 52.4		-38.5 ± 35.1	-64.2 ± 51.8	

Table 3.5: Continued

Gene, SNP, allele (n)	Change android fat, g			Change gynoid fat, g			Change VAT, g			Change SCAT, g		
	High MUFA	Low MUFA	*P*[2]	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*
SREBF1 rs11868035			0.501			0.784			0.867			0.56
A/A (13)	-84.6 ± 52.6	-27.6 ± 70.5		-86.6 ± 61.0	-116.2 ± 77.8		-38.1 ± 41.3	-41 ± 56.5		-44.3 ± 38.1	14.6 ± 55.6	
A/G (54)	-98.7 ± 26.1	-143.1 ± 34.8		-124.4 ± 30.4	-129.1 ± 38.5		-41.5 ± 20.3	-69.3 ± 27.8		-60 ± 18.7	-66.1 ± 27.4	
G/G (34)	-51.6 ± 32.9	-95.7 ± 43.7		-70.3 ± 38.3	-120.3 ± 48.4		-36.3 ± 25.5	-37.9 ± 35.0		-19.1 ± 23.4	-50.4 ± 34.5	
SREBF1 rs2297508			0.465			0.429			0.883			0.569
C/C (16)	-88.6 ± 47.5	-37.7 ± 63.6		-130.6 ± 55.1	-65.9 ± 70.1		-32.9 ± 37.2	-39.8 ± 50.9		-51.5 ± 34.4	0.3 ± 50.2	
C/G (52)	-96.8 ± 26.8	-144.4 ± 35.5		-109.1 ± 31.2	-144.4 ± 39.3		-43.2 ± 20.8	-70.6 ± 28.4		-57.1 ± 19.2	-65.8 ± 28.0	
G/G (33)	-52.8 ± 33.4	-98.9 ± 44.4		-75.5 ± 38.8	-122 ± 49.0		-36.5 ± 25.9	-38.3 ± 35.5		-20.4 ± 23.9	-51.2 ± 35.0	
TCF7L2 rs7903146			0.472			0.408			0.412			0.035
C/C (48)	-85 ± 27.7	-87.3 ± 36.9		-92 ± 33.4	-85.3 ± 41.7		-22.1 ± 21.3	-71.5 ± 29.3		-65.9 ± 19.7	-6.6 ± 28.8	
C/T (46)	-81.3 ± 28.4	-149.9 ± 37.7		-131.7 ± 34.4	-165.9 ± 42.7		-61.6 ± 21.8	-49 ± 30.0		-20.7 ± 20.2	-97 ± 29.5	
T/T (7)	-53.4 ± 71.7	-28 ± 96.3		21.3 ± 82.1	-124.4 ± 106.0		-11.8 ± 57.1	15.6 ± 76.7		-46.9 ± 52.9	-44.3 ± 75.5	

[1]All values are least-squares means and SEMs. Changes were calculated as endpoint-baseline values. The values of the two MUFA diet were averaged due to considerably small difference in MUFA concentrations. n=101 participants. *ADIPOQ*: adiponectin; *ADRB2*: adrenoceptor Beta-2; *APOE*: apolipoprotein E; *FABP1*: liver fatty acid binding protein; *FTO*: fat mass and obesity-associated gene; *IRS1*: Insulin receptor substrate-1; *LIPA*: lipase A, lysosomal acid; *LIPC*: hepatic lipase; *LIPF*: gastric lipase; *LIPG*: endothelial Lipase; low-MUFA: low-MUFA high-saturated fatty acid diet; *LPL*: lipoprotein lipase; MUFA: monounsaturated fatty acids; *PLIN*: perilipin-1; *PPARα*: peroxisome proliferator-activated receptor alpha; *PPARγ*: peroxisome proliferator-activated receptor gamma; SCAT: subcutaneous adipose tissue; *SCD*: stearoyl-CoA desaturase; SNP: single nucleotide polymorphism; *SREBF1*: sterol regulatory element-binding transcription factor 1; *TCF7L2*: transcription factor 7-like 2; VAT: visceral adipose tissue.

[2]SAS PROC MIXED with repeated measure procedure used to assess the effect of gene-MUFA interactions on fat mass changes, using participants as a repeated factor. Treatment, sex, age, and genotype were used as fixed effects. Random effects were treatment sequence, clinical site, and participants. *P* interaction <0.05 was considered significant.

[3]APOE3 is the sum of ε2/ε3 and ε3/ε3 and APOE4 is the sum of ε3/ε4 and ε4/ε4.

The *LPL* rs13702-CC genotype (**Figure 3.2**) was found to reduce VAT (high-MUFA: -216.2 ± 58.6 grams (g); low-MUFA high-SFA: 17.2 ± 81.1 g, p= 0.017), android fat mass (high-MUFA: -267.3 ± 76.4 g; low-MUFA high-SFA: -21.7 ± 102.2 g, p= 0.037), and trunk fat mass (high-MUFA: -1185.1 ± 318.6 g; low-MUFA high-SFA: -269.0 ± 413.3 g, p= 0.044) following high-MUFA consumption compared to the low-MUFA high-SFA diet. Likewise, carriers of the *LPL* rs13702-CC genotype showed trends toward less total fat mass (high-MUFA: -1495.0 ± 385.1 g; low-MUFA high-SFA: -599.9 ± 471.0 g, p= 0.058) and body weight (high-MUFA: -2124.3 ± 584.3 g; low-MUFA high-SFA: -839.9 ± 696.2 g, p= 0.051) following high-MUFA consumption compared to the low-MUFA high-SFA.

The consumption of high-MUFA diets decreased leg fat mass (high-MUFA: -368.4 ± 193.4 g; low-MUFA high-SFA: 281.3 ± 251.6 g, p= 0.029) in the *PPARα* rs6008259-AA homozygotes compared to a low-MUFA high-SFA (**Figure 3.3**). In contrast, leg fat mass increased in these individuals upon consumption of the low-MUFA high-SFA diet. No changes were detected in the carriers of *PPARα* rs6008259-G allele in response to the dietary treatments.

The consumption of low-MUFA high-SFA diet reduced body weight (high-MUFA: -694.24 ± 217.15 g; low-MUFA high-SFA: -1121.3 ± 249.67 g, p= 0.043) in the *FTO* rs9939609-A allele carriers but not upon consumption of high-MUFA diets (**Figure 3.4**). No other gene-diet interactions were observed for the *FTO* rs9939609.

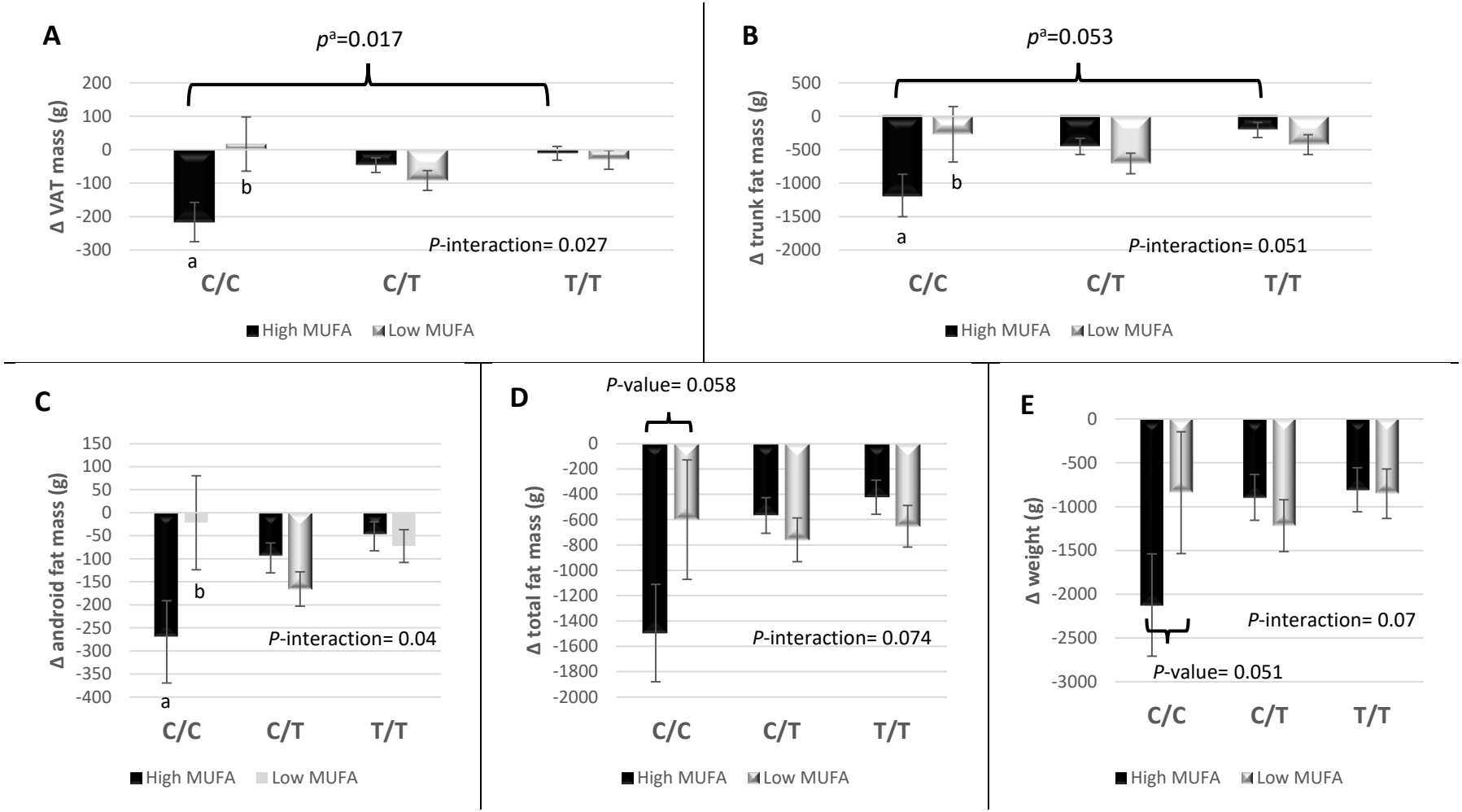

Figure 3.3: *LPL* rs13702 genotypes determine the effect of high versus low MUFA consumption on changes in VAT mass (A), trunk fat mass (B), android fat (C), total fat mass (D), and body weight (E). Total participants=101: n=50 *LPL* rs13702-TT, n=45 *LPL* rs13702-CT, and n=6 *LPL* rs13702-CC. Values are least-squares means ± SEMs. SAS PROC MIXED with repeated measure procedure used to assess the effect of gene-MUFA interactions on fat mass changes, using participants as a repeated factor. Treatment, sex, age, and genotype were used as fixed effects. Random effects were treatment sequence, clinical site, and participants. P<0.05 was considered significant. Labeled bars with different lowercase letters significantly differ. *P*[a]: Indicates greater reduction in VAT mass and trunk fat mass following high-MUFA consumption in CC compared to TT carriers of *LPL* rs13702. Low-MUFA: low-MUFA high-saturated fatty acid diet; *LPL*: lipoprotein lipase; MUFA: monounsaturated fatty acids; VAT: visceral adipose tissue.

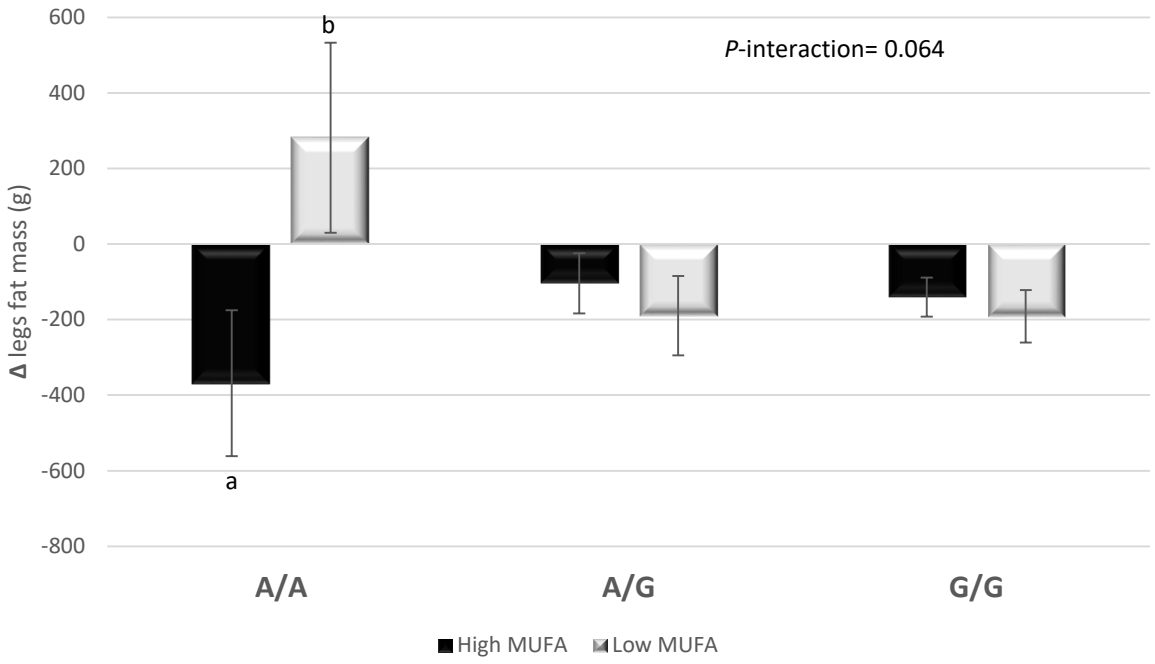

Figure 3.4: *PPARα* rs6008259 genotypes determine the effect of high versus low MUFA consumption on changes in leg fat mass. Total participants=101: n=66 *PPARα* rs6008259-GG, n=30 *PPARα* rs6008259-AG, and n=5 *PPARα* rs6008259-AA. Values are least-squares means ± SEMs. SAS PROC MIXED with repeated measure procedure used to assess the effect of gene-MUFA interactions on fat mass changes, using participants as a repeated factor. Treatment, sex, age, and genotype were used as fixed effects. Random effects were treatment sequence, clinical site, and participants. $P<0.05$ was considered significant. Labeled bars with different lowercase letters significantly differ. Low-MUFA: low-MUFA high-saturated fatty acid diet; MUFA: monounsaturated fatty acids; *PPARα*: peroxisome proliferator-activated receptor alpha.

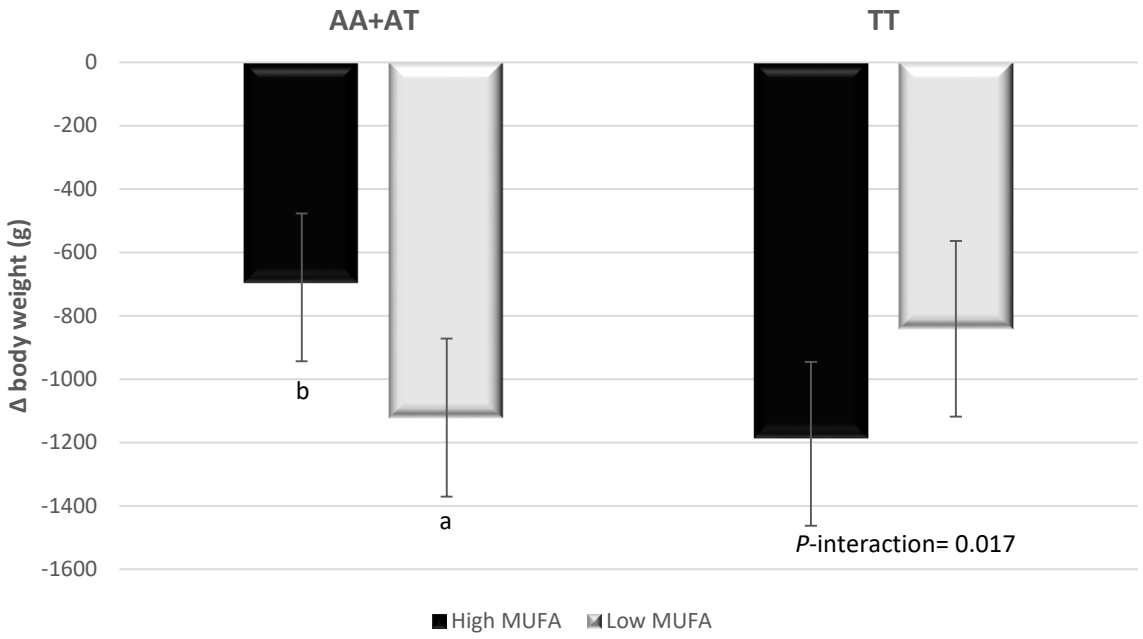

Figure 3.5: *FTO* rs9939609 genotypes determine the effect of high versus low MUFA consumption on changes in body weight. Total participants=101: n=44 *FTO* rs9939609-TT and n=57 *FTO* rs9939609-AA+T. Values are least-squares means ± SEMs. SAS PROC MIXED with repeated measure procedure used to assess the effect of gene-MUFA interactions on fat mass changes, using participants as a repeated factor. Treatment, sex, age, and genotype were used as fixed effects. Random effects were treatment sequence, clinical site, and participants. $P<0.05$ was considered significant. Dominant model (AA+AT versus TT) was analyzed because the simple effect of heterozygous-by-MUFA showed a significant interaction on one or more compartmental fat mass. Labeled bars with different lowercase letters significantly differ. *FTO*: fat mass and obesity-associated gene; Low-MUFA: low-MUFA high-saturated fatty acid diet; MUFA: monounsaturated fatty acids.

SCAT (CC+CG: -67.79 ± 21.00 g; GG: 100.74 ± 62.99 g, *p*= 0.012) and android fat mass (CC+CG: -130.49 ± 26.82 g; GG: 64.72 ± 80.14 g, *p*= 0.022) were elevated in the *ADIPOQ* rs266729-GG homozygotes, but not in C allele carriers, in response to the low-MUFA high-SFA diet (**Figure 3.5**). The consumption of high-MUFA diets protected the *ADIPOQ* rs266729-GG homozygotes from the increase in SCAT that was observed following consumption of the low-MUFA high-SFA diet (high-MUFA: -64.26 ± 42.85 g; low-MUFA high-SFA: 100.74 ± 62.99 g, *p*= 0.037) (**Figure 3.5-A**).

Further, *APOE4* carriers had greater reductions in trunk fat mass (low-MUFA high-SFA: -677.7 ± 133.2 g; high-MUFA: -302.9 ± 106.6 g, *p*= 0.0062) and total fat mass (low-MUFA high-SFA: -862.2 ± 144.7 g; high-MUFA: -497.8 ± 118.6 g, *p*= 0.009) following consumption of the low-MUFA high-SFA diet compared to high-MUFA diets (**Figure 3.6**). Total fat mass in the carriers of GG genotype of the *LIPC* rs6083 (low-MUFA high-SFA: -744.1 ± 283.4 g; high-MUFA: -155.4 ± 229.4 g, *p*= 0.031) (**Figure 3.7-A)** or the *ADRB2* rs1042714 (low-MUFA high-SFA: -1136.7 ± 275.7 g; high-MUFA: -547.7 ± 225.7 g, *p*= 0.028) (**Figure 3.7-B)** was significantly reduced in response to the low-MUFA high-SFA compared to high-MUFA consumption.

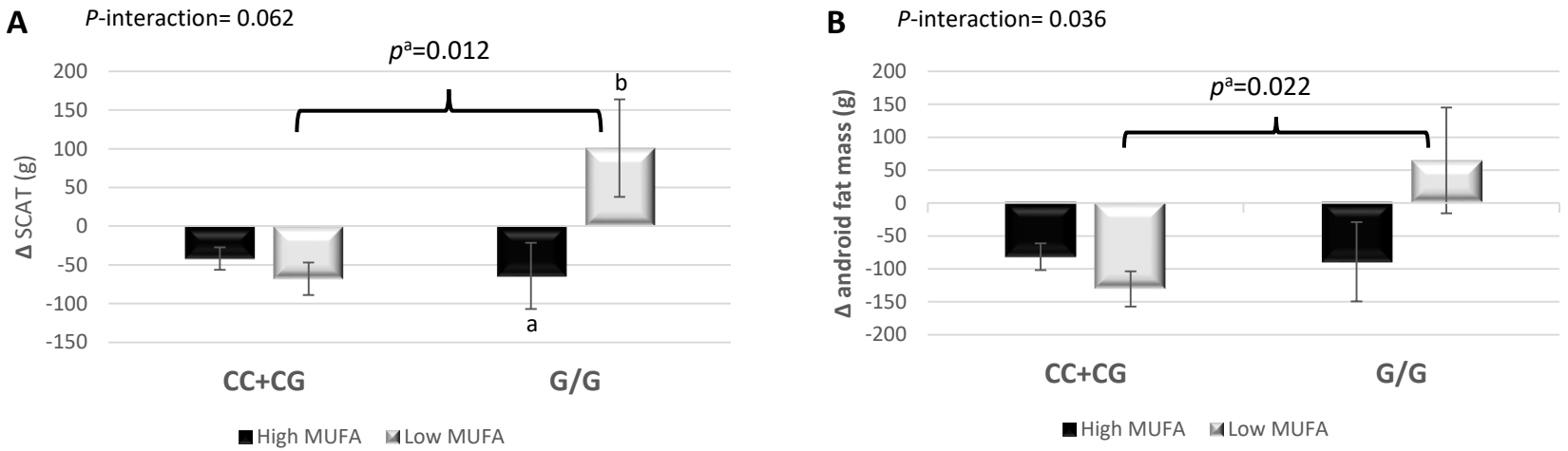

Figure 3.6: *ADIPOQ* rs266729 genotypes determine the effect of high versus low MUFA consumption on changes in SCAT (A) and android fat mass (B). Total participants=101: n=91 *ADIPOQ* rs266729-CC+CG and n=10 *ADIPOQ* rs266729-GG. Values are least-squares means ± SEMs. SAS PROC MIXED with repeated measure procedure used to assess the effect of gene-MUFA interactions on fat mass changes, using participants as a repeated factor. Treatment, sex, age, and genotype were used as fixed effects. Random effects were treatment sequence, clinical site, and participants. $P<0.05$ was considered significant. Labeled bars with different lowercase letters significantly differ. P^a Indicates greater reduction in SCAT mass and android fat mass following low-MUFA consumption in C compared to GG carriers of *ADIPOQ* rs266729. Recessive model (CC+CG versus GG) was analyzed because the simple effect of heterozygous-by-MUFA showed a significant interaction on one or more compartmental fat mass. *ADIPOQ*: adiponectin; Low-MUFA: low-MUFA high-saturated fatty acid diet; MUFA: monounsaturated fatty acids; SCAT: subcutaneous adipose tissue.

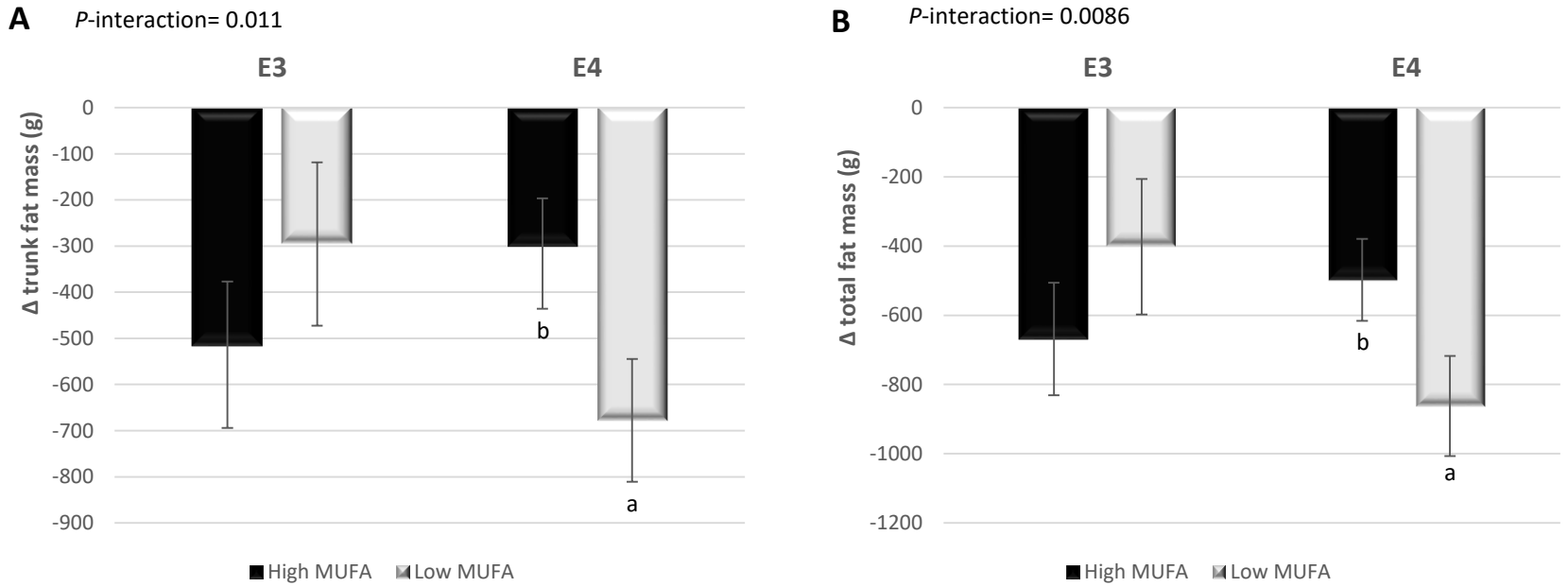

Figure 3.7: *APOE* genotypes determine the effect of high versus low MUFA consumption on changes in trunk fat mass (A) and total fat mass (B). Total participants=101: n=35 *APOE* and n=66 *APOE4*. Values are least-squares means ± SEMs. SAS PROC MIXED with repeated measure procedure used to assess the effect of gene-MUFA interactions on fat mass changes, using participants as a repeated factor. Treatment, sex, age, and genotype were used as fixed effects. Random effects were treatment sequence, clinical site, and participants. $P<0.05$ was considered significant. Labeled bars with different lowercase letters significantly differ. *APOE*: apolipoprotein E; Low-MUFA: low-MUFA high-saturated fatty acid diet; MUFA: monounsaturated fatty acids.

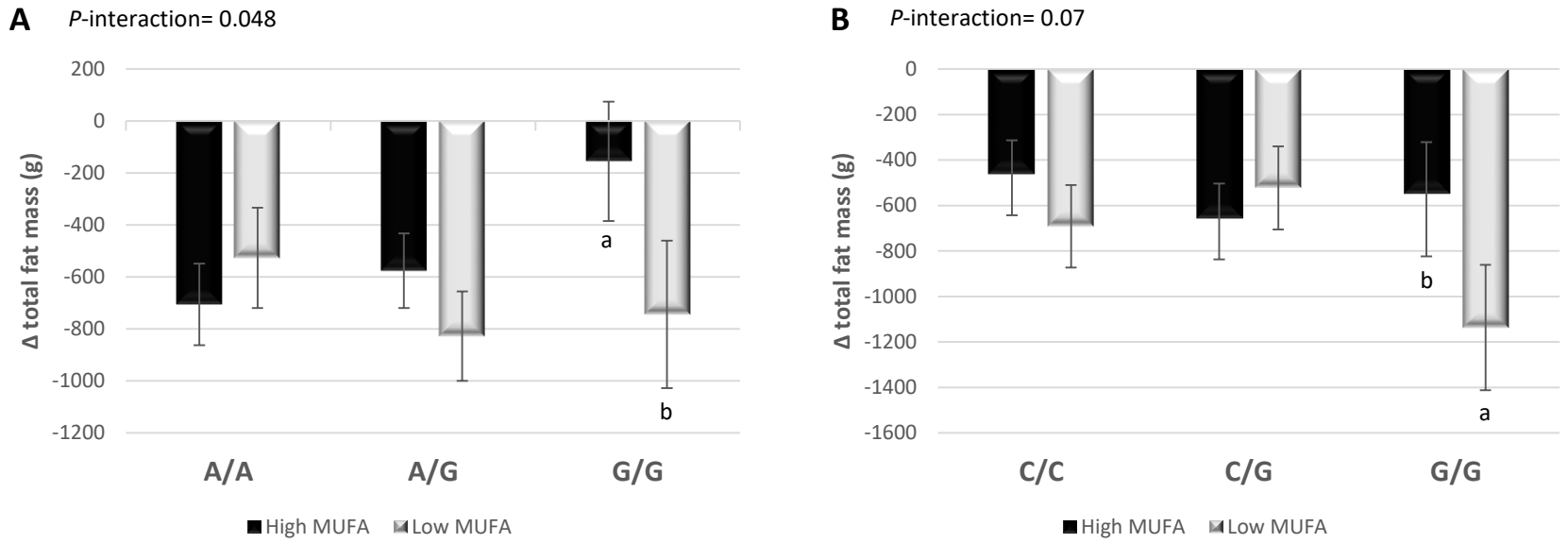

Figure 3.8: Changes in total fat mass in response to high versus low MUFA consumption are determined by *LIPC* rs6083 (A) and *ADRB2* rs1042714 (B) genotypes. Total participants=101: (A) n=37 *LIPC* rs6083-AA, n=47 *LIPC* rs6083-AG, n=17 *LIPC* rs6083-GG and (B) n=42 *ADRB2* rs1042714-CC, n=41 *ADRB2* rs1042714-CG, n=18 *ADRB2* rs1042714-GG. Values are least-squares means ± SEMs. SAS PROC MIXED with repeated measure procedure used to assess the effect of gene-MUFA interactions on fat mass changes, using participants as a repeated factor. Treatment, sex, age, and genotype were used as fixed effects. Random effects were treatment sequence, clinical site, and participants. $P<0.05$ was considered significant. Labeled bars with different lowercase letters significantly differ. *ADRB2*: adrenoceptor Beta 2; Low-MUFA: low-MUFA high-saturated fatty acid diet; *LIPC*: hepatic lipase; MUFA: monounsaturated fatty acids.

3.5 Discussion

The results of the current study indicate that changes in total and compartmental fat mass and body weight in response to dietary fat substitutions are modified by common variants within lipid metabolism-related genes. Variants in *LPL*, *PPARα*, and *ADIPOQ* were associated with lower body fat indices following consumption of high-MUFA diets, whereas variants in *FTO*, *APOE*, *ADRB2*, and *LIPC* were associated with lower body fat indices with low dietary MUFA. These results highlight the genetic contribution to the responsiveness of body fatness to dietary MUFA and may explain our inability to detect significant effects of MUFA consumption on body weight and fat mass compared to the low-MUFA high-SFA diet in spite of the existing evidence (11, 12). From our dietary intervention trial, we cannot conclude on the exact mechanisms of the observed phenomena and we need to refer to future biochemical studies. However, some existing knowledge might help to illuminate the underlying biochemistry.

LPL is the rate-limiting enzyme that catalyzes the hydrolysis of triglycerides in the core of triglyceride-rich lipoprotein constituents as well as facilitating the uptake of FA by adipocytes (27, 28). These functions highlight *LPL* as a candidate gene for obesity. Ma et al. reported no influence of the SNP *LPL* rs13702 under different dietary FA interventions on BMI or waist circumference within two independent populations (29). The C-allele in the functional *LPL* rs13702 is suggested to increase the hydrolytic activity of *LPL* (30, 31); however, to our knowledge, no interactions between *LPL* polymorphisms and dietary FAs to modulate regional fat masses had been reported. The fact that various distinct regions of fat mass and body weight in *LPL* rs13702-CC homozygotes consistently decreased following the high-MUFA diets in an iso-caloric condition provides validity to this interaction. Given the previously proposed *LPL* rs13702-C allele-induced elevation in *LPL* activity, MUFA-rich diets might, therefore, protect

the *LPL* rs13702-CC carriers from an increment in fat mass by the activation of obesity-opposing pathways, such as increasing the activity of hormone-sensitive lipase (32) or elevating the ratio of skeletal muscle to adipose tissue LPL activity (33), which would reduce the propensity of fat deposition.

PPARα a nuclear receptor which is activated by unsaturated fatty acids and a regulator of lipid metabolism (34) is likely involved in such obesity modulating pathways (35). We are not aware of any studies that have evaluated the interactions of *PPARα* polymorphisms and dietary FA on obesity; however, the responsiveness of the *PPARα* rs6008259-AA genotype to dietary FA has been previously reported (36). The opposite effects on leg fat mass observed for the *PPARα* rs6008259-AA homozygotes during high and low MUFA diets could be explained by a differential activation of energy expenditure, despite the iso-caloric diet. However, we caution the fact that only leg fat mass is involved and defer to future studies to validate this observation.

We also report the single observation that carriers of the *FTO* rs9939609-A allele reduced body weight on the low-MUFA high-SFA diet to a greater extent than the high-MUFA diets in an iso-caloric condition. The *FTO* rs9939609-A allele has been associated with a higher body weight at baseline but a greater responsiveness to dietary intervention compared to TT genotype (37-43). Observational studies showed that the *FTO* rs9939609-AA homozygotes who consumed a high-fat diet (39, 40, 44), a high-SFA diet (37), and a low-PUFA: SFA ratio diet (38) had a greater body weight compared to T allele carriers. However, the biochemical roles of the *FTO* gene in the regulation of energy homeostasis, food intake and energy expenditure, as well as FA metabolism are not established (45-47). Therefore, we cannot extrapolate how alleles would determine differential responses to the given dietary FA pattern.

The *ADIPOQ* gene encodes the peptide hormone adiponectin which modulates a number of metabolic processes including lipid oxidation in muscle and liver (48). The *ADIPOQ* rs266729-G allele has been identified as a risk factor for obesity in several studies (49, 50), and was associated with a lower risk for obesity following the consumption of a higher percentage of energy derived from fat (51). The present study shows, despite the controlled iso-caloric diet, that a higher MUFA intake significantly reduced SCAT in the android region among the *ADIPOQ* rs266729-GG homozygotes compared to the low-MUFA high-SFA diet. However, this finding constitutes further refinement of existing obesity associations, specifically since a previous study found no effect of dietary MUFA on the association between the *ADIPOQ* rs266729 and obesity (23). However, we did not assess depot-specific levels of adiponectin or its receptors, leaving the validation of the underlying mechanism to future studies.

APOE gene, encodes apolipoprotein E, mediates the catabolism of the triglyceride-rich lipoprotein particles in an isoform-dependent manner (52, 53). The *APOE4* isoform has been associated with abnormal lipid metabolism and increased risk for several health problems including obesity (54-60) and has previously shown responsiveness to dietary interventions (53, 54, 61). Mice carrying the human *APOE3* allele were heavier on low- and high-fat diets compared to *APOE4* mice (62). The same study found that FA mobilization was lower in *APOE3* than *APOE4* mice, whereas, *APOE4* mice overexpressed proteins involved in fatty acid oxidation in skeletal muscle. Our results add another dimension to the evidence that *APOE* genotypes differentially influence fat mass and distribution, through demonstrating that the level of dietary MUFA modulated fat loss in *APOE4* carriers in an iso-caloric condition. *APOE4* was found to be associated with an increased basal mitochondrial uncoupling and FA oxidation in mice (63), and

this mechanism might very well be modulated by the degree of dietary MUFAs, especially given that different dietary MUFA could increase fat oxidation and thermogenesis (7).

Our observations that total fat mass decreased to a higher degree in the homozygotes for SNPs *LIPC* rs6083-GG and *ADRB2* rs1042714-GG following the low-MUFA high-SFA diet are unprecedented. The controlled iso-caloric design of this study underscores these findings. The *LIPC* gene encodes the enzyme hepatic lipase which has a dual function of triglyceride lipolysis from lipoprotein particles and as a ligand factor for receptor-mediated lipoprotein uptake (64). *LIPC* contributes to the regulation of energy homeostasis (65), and its activity was positively correlated with body fat, specifically intra-abdominal fat (65-68). The *ADRB2* gene encodes the β2 adrenoceptors which are a major lipolytic receptor in adipocytes that regulates thermogenesis and lipid mobilization from adipocytes, and therefore, regulates energy balance (69, 70). The *ADRB2* rs1042714 G-allele has repeatedly shown to be a risk factor for adiposity in different ethnic groups (71-75) but induced a greater reduction in body weight in response to energy restricted diets in women (69) and men (76). No evidence for possible mechanisms explaining the differential regulation of these SNPs by MUFA can be found in the existing literature. We, therefore, caution that the validity of these findings should be the focus of future investigations.

Unlike previous studies that focused on a particular SNP or gene, the current study provides emerging evidence for several diet-gene interactions modifying fat mass accumulations at high- and low-MUFA consumption. Assessing adiposity using DXA scanning provided a comprehensive assessment of the effect of these SNPs on total and regional adiposity. The crossover design with a controlled, iso-caloric dietary intervention represents another strength of this study, which eliminated a range of confounders that might be inherent with free-living and/or parallel study designs. However, one important limitation of this study is that we did not

apply stringent control for multiple testing, which may lead to a potential overstatement of our findings. Nevertheless, nine of the tested SNPs in this study have been previously reported to interaction with dietary fat on obesity, therefore, we consider this as a replication study for these SNPs, which would reduce the need to apply multiple testing. The mixed ethnicity of this study population may be perceived as a limitation, yet, it might provide generalizability of the current findings.

In the current study, we report the contribution of common variants in *LPL*, *FTO*, *PPARα*, *ADIPOQ*, *APOE*, *LIPC*, and *ADRB2* to changes in body fatness in response to dietary MUFA. These changes in body fat were observed regardless of the controlled iso-caloric scenario. Although the observed changes in total and compartmental fat mass in response to gene-MUFA interactions were small over 6 weeks, their statistical significance may indicate a potential clinical effect in weight reduction/maintenance regimens over prolonged periods.

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Authors' contributions: The authors' responsibilities were as follows: SSH, JS, XC, PC, VG, JMG, DP, AW, SC, PZ, and KJB were involved in the clinical trial conduction and data collection; SSH performed the laboratory work, data interpretation, and wrote the manuscript; PE contributed expertise for genotyping studies; PWC, BL, DJAJ, CT, SGW, PMKE, and PJHJ designed the project and supervised the study. All authors critically reviewed the manuscript and read and approved the final version.

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3.6 References

1. Liu X, Kris-Etherton PM, West SG, Lamarche B, Jenkins DJ, Fleming JA, McCrea CE, Pu S, Couture P, Connelly PW, et al. Effects of canola and high-oleic-acid canola oils on abdominal fat mass in individuals with central obesity. Obesity (Silver Spring) 2016;24(11):2261-8. doi: 10.1002/oby.21584.

2. Alves RD, Moreira AP, Macedo VS, de Cassia Goncalves Alfenas R, Bressan J, Mattes R, Costa NM. Regular intake of high-oleic peanuts improves fat oxidation and body composition in overweight/obese men pursuing a energy-restricted diet. Obesity (Silver Spring) 2014;22(6):1422-9. doi: 10.1002/oby.20746.

3. Summers LK, Fielding BA, Bradshaw HA, Ilic V, Beysen C, Clark ML, Moore NR, Frayn KN. Substituting dietary saturated fat with polyunsaturated fat changes abdominal fat distribution and improves insulin sensitivity. Diabetologia 2002;45(3):369-77. doi: 10.1007/s00125-001-0768-3.

4. Mumme K, Stonehouse W. Effects of medium-chain triglycerides on weight loss and body composition: a meta-analysis of randomized controlled trials. J Acad Nutr Diet 2015;115(2):249-63. doi: 10.1016/j.jand.2014.10.022.

5. Mennella I, Savarese M, Ferracane R, Sacchi R, Vitaglione P. Oleic acid content of a meal promotes oleoylethanolamide response and reduces subsequent energy intake in humans. Food Funct 2015;6(1):204-10. doi: 10.1039/c4fo00697f.

6. Stevenson JL, Clevenger HC, Cooper JA. Hunger and satiety responses to high-fat meals of varying fatty acid composition in women with obesity. Obesity (Silver Spring) 2015;23(10):1980-6. doi: 10.1002/oby.21202.

7. Krishnan S, Cooper JA. Effect of dietary fatty acid composition on substrate utilization and body weight maintenance in humans. Eur J Nutr 2014;53(3):691-710. doi: 10.1007/s00394-013-0638-z.

8. Kien CL, Bunn JY. Gender alters the effects of palmitate and oleate on fat oxidation and energy expenditure. Obesity (Silver Spring) 2008;16(1):29-33. doi: 10.1038/oby.2007.13.

9. Piers LS, Walker KZ, Stoney RM, Soares MJ, O'Dea K. The influence of the type of dietary fat on postprandial fat oxidation rates: monounsaturated (olive oil) vs saturated fat (cream). Int J Obes Relat Metab Disord 2002;26(6):814-21. doi: 10.1038/sj.ijo.0801993.

10. Kien CL, Bunn JY, Tompkins CL, Dumas JA, Crain KI, Ebenstein DB, Koves TR, Muoio DM. Substituting dietary monounsaturated fat for saturated fat is associated with increased daily physical activity and resting energy expenditure and with changes in mood. Am J Clin Nutr 2013;97(4):689-97. doi: 10.3945/ajcn.112.051730.

11. Piers LS, Walker KZ, Stoney RM, Soares MJ, O'Dea K. Substitution of saturated with monounsaturated fat in a 4-week diet affects body weight and composition of overweight and obese men. Br J Nutr 2003;90(3):717-27.

12. Gillingham LG, Robinson KS, Jones PJ. Effect of high-oleic canola and flaxseed oils on energy expenditure and body composition in hypercholesterolemic subjects. Metabolism 2012;61(11):1598-605. doi: 10.1016/j.metabol.2012.04.016.

13. Andersen MK, Sandholt CH. Recent progress in the understanding of obesity: contributions of genome-wide association studies. Curr Obes Rep 2015;4(4):401-10. doi: 10.1007/s13679-015-0173-8.

14. Fall T, Ingelsson E. Genome-wide association studies of obesity and metabolic syndrome. Mol Cell Endocrinol 2014;382(1):740-57. doi: 10.1016/j.mce.2012.08.018.

15. Moreno-Aliaga MJ, Santos JL, Marti A, Martinez JA. Does weight loss prognosis depend on genetic make-up? Obes Rev 2005;6(2):155-68. doi: 10.1111/j.1467-789X.2005.00180.x.

16. Garaulet M, Smith CE, Hernandez-Gonzalez T, Lee YC, Ordovas JM. PPARgamma Pro12Ala interacts with fat intake for obesity and weight loss in a behavioural treatment based on the Mediterranean diet. Mol Nutr Food Res 2011;55(12):1771-9. doi: 10.1002/mnfr.201100437.

17. Memisoglu A, Hu FB, Hankinson SE, Manson JE, De Vivo I, Willett WC, Hunter DJ. Interaction between a peroxisome proliferator-activated receptor gamma gene polymorphism and dietary fat intake in relation to body mass. Hum Mol Genet 2003;12(22):2923-9. doi: 10.1093/hmg/ddg318.

18. Rosado EL, Bressan J, Martinez JA, Marques-Lopes I. Interactions of the PPARgamma2 polymorphism with fat intake affecting energy metabolism and nutritional outcomes in obese women. Ann Nutr Metab 2010;57(3-4):242-50. doi: 10.1159/000322187.

19. Franks PW, Jablonski KA, Delahanty L, Hanson RL, Kahn SE, Altshuler D, Knowler WC, Florez JC. The Pro12Ala variant at the peroxisome proliferator-activated receptor gamma gene and change in obesity-related traits in the Diabetes Prevention Program. Diabetologia 2007;50(12):2451-60. doi: 10.1007/s00125-007-0826-6.

20. Luan J, Browne PO, Harding AH, Halsall DJ, O'Rahilly S, Chatterjee VK, Wareham NJ. Evidence for gene-nutrient interaction at the PPARgamma locus. Diabetes 2001;50(3):686-9.

21. Smith CE, Ngwa J, Tanaka T, Qi Q, Wojczynski MK, Lemaitre RN, Anderson JS, Manichaikul A, Mikkila V, van Rooij FJ, et al. Lipoprotein receptor-related protein 1 variants and dietary fatty acids: meta-analysis of European origin and African American studies. Int J Obes (Lond) 2013;37(9):1211-20. doi: 10.1038/ijo.2012.215.

22. Rosado EL, Bressan J, Hernandez JA, Martins MF, Cecon PR. [Effect of diet and PPARgamma2 and beta2-adrenergic receptor genes on energy metabolism and body composition in obese women]. Nutr Hosp 2006;21(3):317-31.

23. Warodomwichit D, Shen J, Arnett DK, Tsai MY, Kabagambe EK, Peacock JM, Hixson JE, Straka RJ, Province MA, An P, et al. ADIPOQ polymorphisms, monounsaturated fatty acids, and obesity risk: the GOLDN study. Obesity (Silver Spring) 2009;17(3):510-7. doi: 10.1038/oby.2008.583.

24. Garaulet M, Smith CE, Gomez-Abellan P, Ordovas-Montanes M, Lee YC, Parnell LD, Arnett DK, Ordovas JM. REV-ERB-ALPHA circadian gene variant associates with obesity in two independent populations: Mediterranean and North American. Mol Nutr Food Res 2014;58(4):821-9. doi: 10.1002/mnfr.201300361.

25. Miazgowski T, Krzyzanowska-Swiniarska B, Dziwura-Ogonowska J, Widecka K. The associations between cardiometabolic risk factors and visceral fat measured by a new dual-energy X-ray absorptiometry-derived method in lean healthy Caucasian women. Endocrine 2014;47(2):500-5. doi: 10.1007/s12020-014-0180-7.

26. Kaul S, Rothney MP, Peters DM, Wacker WK, Davis CE, Shapiro MD, Ergun DL. Dual-energy X-ray absorptiometry for quantification of visceral fat. Obesity (Silver Spring) 2012;20(6):1313-8. doi: 10.1038/oby.2011.393.

27. Wang H, Eckel RH. Lipoprotein lipase: from gene to obesity. Am J Physiol Endocrinol Metab 2009;297(2):E271-88. doi: 10.1152/ajpendo.90920.2008.

28. Mead JR, Irvine SA, Ramji DP. Lipoprotein lipase: structure, function, regulation, and role in disease. J Mol Med (Berl) 2002;80(12):753-69. doi: 10.1007/s00109-002-0384-9.

29. Ma Y, Tucker KL, Smith CE, Lee YC, Huang T, Richardson K, Parnell LD, Lai CQ, Young KL, Justice AE, et al. Lipoprotein lipase variants interact with polyunsaturated fatty acids for obesity traits in women: replication in two populations. Nutr Metab Cardiovasc Dis 2014;24(12):1323-9. doi: 10.1016/j.numecd.2014.07.003.

30. Richardson K, Nettleton JA, Rotllan N, Tanaka T, Smith CE, Lai CQ, Parnell LD, Lee YC, Lahti J, Lemaitre RN, et al. Gain-of-function lipoprotein lipase variant rs13702 modulates lipid traits through disruption of a microRNA-410 seed site. Am J Hum Genet 2013;92(1):5-14. doi: 10.1016/j.ajhg.2012.10.020.

31. Goodarzi MO, Wong H, Quinones MJ, Taylor KD, Guo X, Castellani LW, Antoine HJ, Yang H, Hsueh WA, Rotter JI. The 3' untranslated region of the lipoprotein lipase gene: haplotype structure and association with post-heparin plasma lipase activity. J Clin Endocrinol Metab 2005;90(8):4816-23. doi: 10.1210/jc.2005-0389.

32. Rivellese AA, Giacco R, Annuzzi G, De Natale C, Patti L, Di Marino L, Minerva V, Costabile G, Santangelo C, Masella R, et al. Effects of monounsaturated vs. saturated fat on postprandial lipemia and adipose tissue lipases in type 2 diabetes. Clin Nutr 2008;27(1):133-41. doi: 10.1016/j.clnu.2007.07.005.

33. Simsolo RB, Ong JM, Kern PA. The regulation of adipose tissue and muscle lipoprotein lipase in runners by detraining. J Clin Invest 1993;92(5):2124-30. doi: 10.1172/jci116813.

34. Contreras AV, Torres N, Tovar AR. PPAR-alpha as a key nutritional and environmental sensor for metabolic adaptation. Adv Nutr 2013;4(4):439-52. doi: 10.3945/an.113.003798.

35. Blaschke F, Takata Y, Caglayan E, Law RE, Hsueh WA. Obesity, peroxisome proliferator-activated receptor, and atherosclerosis in type 2 diabetes. Arterioscler Thromb Vasc Biol 2006;26(1):28-40. doi: 10.1161/01.atv.0000191663.12164.77.

36. Volcik KA, Nettleton JA, Ballantyne CM, Boerwinkle E. Peroxisome proliferator-activated receptor [alpha] genetic variation interacts with n-6 and long-chain n-3 fatty acid intake to affect total cholesterol and LDL-cholesterol concentrations in the Atherosclerosis Risk in Communities Study. Am J Clin Nutr 2008;87(6):1926-31.

37. Corella D, Arnett DK, Tucker KL, Kabagambe EK, Tsai M, Parnell LD, Lai CQ, Lee YC, Warodomwichit D, Hopkins PN, et al. A high intake of saturated fatty acids strengthens the association between the fat mass and obesity-associated gene and BMI. J Nutr 2011;141(12):2219-25. doi: 10.3945/jn.111.143826.

38. Phillips CM, Kesse-Guyot E, McManus R, Hercberg S, Lairon D, Planells R, Roche HM. High dietary saturated fat intake accentuates obesity risk associated with the fat mass and obesity-associated gene in adults. J Nutr 2012;142(5):824-31. doi: 10.3945/jn.111.153460.

39. Lappalainen T, Lindstrom J, Paananen J, Eriksson JG, Karhunen L, Tuomilehto J, Uusitupa M. Association of the fat mass and obesity-associated (FTO) gene variant (rs9939609) with dietary intake in the Finnish Diabetes Prevention Study. Br J Nutr 2012;108(10):1859-65. doi: 10.1017/s0007114511007410.

40. Sonestedt E, Roos C, Gullberg B, Ericson U, Wirfalt E, Orho-Melander M. Fat and carbohydrate intake modify the association between genetic variation in the FTO genotype and obesity. Am J Clin Nutr 2009;90(5):1418-25. doi: 10.3945/ajcn.2009.27958.

41. Woehning A, Schultz JH, Roeder E, Moeltner A, Isermann B, Nawroth PP, Wolfrum C, Rudofsky G. The A-allele of the common FTO gene variant rs9939609 complicates weight maintenance in severe obese patients. Int J Obes (Lond) 2013;37(1):135-9. doi: 10.1038/ijo.2012.14.

42. Razquin C, Martinez JA, Martinez-Gonzalez MA, Bes-Rastrollo M, Fernandez-Crehuet J, Marti A. A 3-year intervention with a Mediterranean diet modified the association between the rs9939609 gene variant in FTO and body weight changes. Int J Obes (Lond) 2010;34(2):266-72. doi: 10.1038/ijo.2009.233.

43. de Luis DA, Aller R, Conde R, Izaola O, Gonzalez Sagrado M, Castrodeza Sanz J. The rs9939609 gene variant in FTO modified the metabolic response of weight loss after a 3-month intervention with a hypocaloric diet. J Investig Med 2013;61(1):22-6. doi: 10.2310/JIM.0b013e318276161d.

44. Moleres A, Ochoa MC, Rendo-Urteaga T, Martinez-Gonzalez MA, Azcona San Julian MC, Martinez JA, Marti A. Dietary fatty acid distribution modifies obesity risk linked to the

rs9939609 polymorphism of the fat mass and obesity-associated gene in a Spanish case-control study of children. Br J Nutr 2012;107(4):533-8. doi: 10.1017/s0007114511003424.

45. Apalasamy YD, Ming MF, Rampal S, Bulgiba A, Mohamed Z. Genetic association of SNPs in the FTO gene and predisposition to obesity in Malaysian Malays. Braz J Med Biol Res 2012;45(12):1119-26.

46. Prakash J, Mittal B, Srivastava A, Awasthi S, Srivastava N. Association of FTO rs9939609 SNP with obesity and obesity- associated phenotypes in a North Indian population. Oman Med J 2016;31(2):99-106. doi: 10.5001/omj.2016.20.

47. Fawcett KA, Barroso I. The genetics of obesity: FTO leads the way. Trends Genet 2010;26(6):266-74. doi: 10.1016/j.tig.2010.02.006.

48. Nigro E, Scudiero O, Monaco ML, Palmieri A, Mazzarella G, Costagliola C, Bianco A, Daniele A. New insight into adiponectin role in obesity and obesity-related diseases. Biomed Res Int 2014;2014:658913. doi: 10.1155/2014/658913.

49. Park JW, Park J, Jee SH. ADIPOQ gene variants associated with susceptibility to obesity and low serum adiponectin levels in healthy Koreans. Epidemiol Health 2011;33:e2011003. doi: 10.4178/epih/e2011003.

50. Karmelic I, Lovric J, Bozina T, Ljubic H, Vogrinc Z, Bozina N, Sertic J. Adiponectin level and gene variability are obesity and metabolic syndrome markers in a young population. Arch Med Res 2012;43(2):145-53. doi: 10.1016/j.arcmed.2012.02.004.

51. Santos JL, Boutin P, Verdich C, Holst C, Larsen LH, Toubro S, Dina C, Saris WH, Blaak EE, Hoffstedt J, et al. Genotype-by-nutrient interactions assessed in European obese women. A case-only study. Eur J Nutr 2006;45(8):454-62. doi: 10.1007/s00394-006-0619-6.

52. Moreno JA, Perez-Jimenez F, Marin C, Gomez P, Perez-Martinez P, Moreno R, Bellido C, Fuentes F, Lopez-Miranda J. The effect of dietary fat on LDL size is influenced by apolipoprotein E genotype in healthy subjects. J Nutr 2004;134(10):2517-22.

53. Saito M, Eto M, Nitta H, Kanda Y, Shigeto M, Nakayama K, Tawaramoto K, Kawasaki F, Kamei S, Kohara K, et al. Effect of apolipoprotein E4 allele on plasma LDL cholesterol response to diet therapy in type 2 diabetic patients. Diabetes Care 2004;27(6):1276-80.

54. Paula RS, Souza VC, Benedet AL, Souza ER, Toledo JO, Moraes CF, Gomes L, Alho CS, Cordova C, Nobrega OT. Dietary fat and apolipoprotein genotypes modulate plasma lipoprotein levels in Brazilian elderly women. Mol Cell Biochem 2010;337(1-2):307-15. doi: 10.1007/s11010-009-0313-0.

55. Chouinard-Watkins R, Plourde M. Fatty acid metabolism in carriers of apolipoprotein E epsilon 4 allele: is it contributing to higher risk of cognitive decline and coronary heart disease? Nutrients 2014;6(10):4452-71. doi: 10.3390/nu6104452.

56. Alharbi KK, Syed R, Alharbi FK, Khan IA. Association of apolipoprotein E polymorphism with impact on overweight university pupils. Genet Test Mol Biomarkers 2017;21(1):53-7. doi: 10.1089/gtmb.2016.0190.

57. Kolovou GD, Anagnostopoulou KK, Kostakou P, Giannakopoulou V, Mihas C, Hatzigeorgiou G, Vasiliadis IK, Mikhailidis DP, Cokkinos DV. Apolipoprotein E gene polymorphism and obesity status in middle-aged men with coronary heart disease. In Vivo 2009;23(1):33-9.

58. Zarkesh M, Daneshpour MS, Faam B, Hedayati M, Azizi F. Is there any association of apolipoprotein E gene polymorphism with obesity status and lipid profiles? Tehran Lipid and Glucose Study (TLGS). Gene 2012;509(2):282-5. doi: https://doi.org/10.1016/j.gene.2012.07.048.

59. Tabatabaei-Malazy O, Fakhrzadeh H, Qorbani M, Amiri P, Larijani B, Tavakkoly-Bazzaz J, Amoli MM. Apolipoprotein E gene polymorphism and its effect on anthropometric measures in normoglycemic subjects and type 2 diabetes. J Diabetes Metab Disord 2012;11(1):18. doi: 10.1186/2251-6581-11-18.

60. Rodriguez-Carmona Y, Perez-Rodriguez M, Gamez-Valdez E, Lopez-Alavez FJ, Hernandez-Armenta CI, Vega-Monter N, Leyva-Garcia G, Monge-Cazares T, Barrera Valencia D, Balderas Monroy M, et al. Association between apolipoprotein E variants and obesity-related traits in Mexican school children. J Nutrigenet Nutrigenomics 2014;7(4-6):243-51. doi: 10.1159/000381345.

61. Chouinard-Watkins R, Conway V, Minihane AM, Jackson KG, Lovegrove JA, Plourde M. Interaction between BMI and APOE genotype is associated with changes in the plasma long-chain-PUFA response to a fish-oil supplement in healthy participants. Am J Clin Nutr 2015;102(2):505-13. doi: 10.3945/ajcn.114.103507.

62. Huebbe P, Dose J, Schloesser A, Campbell G, Gluer CC, Gupta Y, Ibrahim S, Minihane AM, Baines JF, Nebel A, et al. Apolipoprotein E (APOE) genotype regulates body weight and fatty acid utilization-Studies in gene-targeted replacement mice. Mol Nutr Food Res 2015;59(2):334-43. doi: 10.1002/mnfr.201400636.

63. Slim KE, Vauzour D, Tejera N, Voshol PJ, Cassidy A, Minihane AM. The effect of dietary fish oil on weight gain and insulin sensitivity is dependent on APOE genotype in humanized targeted replacement mice. Faseb j 2017;31(3):989-97. doi: 10.1096/fj.201600921RR.

64. Ordovas JM, Corella D, Demissie S, Cupples LA, Couture P, Coltell O, Wilson PW, Schaefer EJ, Tucker KL. Dietary fat intake determines the effect of a common polymorphism in the hepatic lipase gene promoter on high-density lipoprotein metabolism: evidence of a strong dose effect in this gene-nutrient interaction in the Framingham Study. Circulation 2002;106(18):2315-21.

65. Wang H, Zhang D, Ling J, Lu W, Zhang S, Zhu Y, Lai M. Gender specific effect of LIPC C-514T polymorphism on obesity and relationship with plasma lipid levels in Chinese children. J Cell Mol Med 2015;19(9):2296-306. doi: 10.1111/jcmm.12663.

66. Farahani P, Fisler JS, Wong H, Diament AL, Yi N, Warden CH. Reciprocal hemizygosity analysis of mouse hepatic lipase reveals influence on obesity. Obes Res 2004;12(2):292-305. doi: 10.1038/oby.2004.37.

67. Carr MC, Brunzell JD, Deeb SS. Ethnic differences in hepatic lipase and HDL in Japanese, black, and white Americans: role of central obesity and LIPC polymorphisms. J Lipid Res 2004;45(3):466-73. doi: 10.1194/jlr.M300295-JLR200.

68. Sibley SD, Palmer JP, Hirsch IB, Brunzell JD. Visceral obesity, hepatic lipase activity, and dyslipidemia in type 1 diabetes. J Clin Endocrinol Metab 2003;88(7):3379-84. doi: 10.1210/jc.2002-021693.

69. Ruiz JR, Larrarte E, Margareto J, Ares R, Labayen I. Role of beta(2)-adrenergic receptor polymorphisms on body weight and body composition response to energy restriction in obese women: preliminary results. Obesity (Silver Spring) 2011;19(1):212-5. doi: 10.1038/oby.2010.130.

70. Tan LJ, Zhu H, He H, Wu KH, Li J, Chen XD, Zhang JG, Shen H, Tian Q, Krousel-Wood M, et al. Replication of 6 obesity genes in a meta-analysis of genome-wide association studies from diverse ancestries. PLoS One 2014;9(5):e96149. doi: 10.1371/journal.pone.0096149.

71. Lange LA, Norris JM, Langefeld CD, Nicklas BJ, Wagenknecht LE, Saad MF, Bowden DW. Association of adipose tissue deposition and beta-2 adrenergic receptor variants: the IRAS family study. Int J Obes (Lond) 2005;29(5):449-57. doi: 10.1038/sj.ijo.0802883.

72. Jalba MS, Rhoads GG, Demissie K. Association of codon 16 and codon 27 beta 2-adrenergic receptor gene polymorphisms with obesity: a meta-analysis. Obesity (Silver Spring) 2008;16(9):2096-106.

73. Corbalan MS, Marti A, Forga L, Martinez-Gonzalez MA, Martinez JA. Beta(2)-adrenergic receptor mutation and abdominal obesity risk: effect modification by gender and HDL-cholesterol. Eur J Nutr 2002;41(3):114-8. doi: 10.1007/s00394-002-0363-5.

74. Rauhio A, Uusi-Rasi K, Nikkari ST, Kannus P, Sievanen H, Kunnas T. Association of the FTO and ADRB2 genes with body composition and fat distribution in obese women. Maturitas 2013;76(2):165-71. doi: 10.1016/j.maturitas.2013.07.004.

75. Zhang H, Wu J, Yu L. Association of Gln27Glu and Arg16Gly polymorphisms in Beta2-adrenergic receptor gene with obesity susceptibility: a meta-analysis. PLoS One 2014;9(6):e100489. doi: 10.1371/journal.pone.0100489.

76. Szendrei B, Gonzalez-Lamuno D, Amigo T, Wang G, Pitsiladis Y, Benito PJ, Gomez-Candela C, Calderon FJ, Cupeiro R. Influence of ADRB2 Gln27Glu and ADRB3 Trp64Arg

polymorphisms on body weight and body composition changes after a controlled weight-loss intervention. Appl Physiol Nutr Metab 2016;41(3):307-14. doi: 10.1139/apnm-2015-0425.

BRIDGE TO CHAPTER IV

Chapter II and Chapter III demonstrated that the response of body weight and body fat mass changes to dietary MUFA can be modified by genetic polymorphisms. Some of these polymorphisms may control the splicing of genes and, thereafter, their expression levels. Exploring the effects of dietary FA composition on the abundance of obesity-related genes is important for understanding the mechanisms underlying the metabolic influence of fatty acids. There is a dearth of evidence concerning the effect of MUFA on expression levels of obesity-related genes in humans. Chapter IV comprises a manuscript that reports an exploratory analysis of the influence of high dietary MUFA on the expression of candidate obesity-related genes and transcription factors. Shatha Hammad participated in study coordination and data collection at RCFFN, processed body composition data for all participants, handled relative gene expression calculations, performed the statistical analyses, and wrote the manuscript.

CHAPTER IV

MANUSCRIPT 3

Dietary monounsaturated fatty acids modulate CPT1B expression level in peripheral whole blood cells of subjects with abdominal obesity: A randomized controlled trial

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4.1 Abstract

Background: Current evidence is increasingly in support of a beneficial role of monounsaturated fatty acid (MUFA) on fat deposition and distribution. Evaluating the influence of MUFA consumption on expression levels of obesity-related genes in humans will help understand the biological mechanism underlying the obesity opposing effects of MUFA.

Objective: Exploring the effect of MUFA-rich oil consumption on expression levels of several obesity-related genes and transcription factors.

Method: In a randomized, crossover, iso-caloric, controlled-feeding multicentre trial, participants consumed one of three oils (20% of total energy) for six weeks, separated by a four-twelve week washout. Oils included regular canola oil (RCO), high-oleic canola oil (HOCO), and a low-MUFA high-saturated fatty acid control oil blend. Expression levels of 19 selected obesity-associated genes and transcription factors were analyzed in whole blood samples of 42 participants with abdominal obesity using quantitative PCR.

Results: Relative expression level of *CPT1B* after RCO consumption compared to control treatment was elevated by 17%, and was significantly (p= 0.0091) higher than its expression level following consumption of HOCO. Consumption of MUFA-rich oils did not modulate expression levels of the other selected genes and transcription factors. No significant treatment effect was observed on changes in body weight or body fat mass. However, following RCO consumption, moderate negative correlations were observed between *SCD1* relative expression and change in gynoid fat mass (r^2= -0.40, p=0.008) as well as *PLIN1* relative expression and change in subcutaneous fat mass (r^2= -0.52, p=0.002). No significant correlation was found between body fat mass and expression levels upon HOCO consumption.

Conclusion: Different levels of dietary MUFA modified expression level of *CPT1B* and the correlations of *SCD1* and *PLIN1* with body fat mass. These findings might help illuminate the underlying mechanism underpinning the beneficial effects of MUFA consumption on energy balance. The trial was registered at clinicaltrials.gov as NCT02029833.

4.2 Introduction

Dietary fatty acid composition is gaining increasing attention regarding its influence in energy and weight balance, where various fatty acids differ in their obesity-inducing effects (1-3). Growing evidence demonstrates the role of dietary monounsaturated fatty acids (MUFA) in increasing fat oxidation, diet-induced thermogenesis (2, 4, 5), resting energy expenditure (6), and promoting weight loss (1, 7) to a greater extent compared to saturated fatty acids (SFA). Recently, we found significant reduction in android fat mass following the consumption of two MUFA-rich oils, canola oil and a high-oleic canola oil, compared to a high polyunsaturated fatty acids (PUFA) flaxseed/safflower oil using a controlled iso-caloric diet study design in participants with abdominal obesity (8).

Differences in the obesogenic effect of various fatty acids might be attributed to variations in their influence on expression levels of several key regulatory and functional genes involved in lipogenesis and adipogenesis (9-12). For instance, docosahexaenoic acid reduced the activation of cannabinoid receptor-1 by altering the expression of endocannabinoid system-associated genes and resulted in a lower fat accretion in mice compared to safflower oil-enriched diet (13). Unsaturated fatty acids alter energy balance by acting as physiological ligands of the uncoupling protein-2 (*UCP2*); the expression of which was upregulated by *in vitro* PUFA and MUFA stimulation compared to SFA (14). Further, MUFA consumption within an iso-caloric diet was found to prevent the reduction in postprandial adiponectin gene expression levels in peripheral tissue and induced a lower central fat deposition compared to a high-carbohydrate diet in insulin resistant subjects (15). Exploring the effects of dietary fatty acid composition on the abundance of obesity-related genes is important in improving our understanding of the mechanisms underlying the metabolic influence of fatty acids.

Little has been reported concerning the effect of MUFA on expression levels of obesity-related genes. Here, in this exploratory analysis, the objective was to investigate the influence of high dietary MUFA from canola oils on the expression of candidate obesity-related genes and transcription factors in whole blood cells.

4.3 Subjects and Methods

4.3.1 Study design and population

The Canola Oil Multi-center Intervention Trial II (COMIT II) was a randomized, controlled, double-blind, crossover study designed to investigate the effect of high-oleic canola oil (HOCO) and regular canola oil (RCO) versus a low-MUFA high-SFA oil blend (Control) on body composition.

Participants aged 20-65 years were included if they had abdominal obesity defined as a waist circumference of ≥94 cm in men and ≥80 cm in women, in addition to at least one of the following metabolic syndrome criteria; fasting blood glucose of ≥ 5.6 mmol/L, triglycerides ≥1.7 mmol/L, high-density lipoprotein-cholesterol <1 mmol/L (men) or <1.3 mmol/L (women), and blood pressure ≥130 mmHg (systolic) and/or ≥85 mmHg (diastolic). Individuals were excluded if they possess uncontrolled thyroid disease, kidney disease, diabetes, or liver disease. Volunteers were not permitted to participate in the study if they were current smokers, consuming > 2 alcoholic beverages per day, taking medication known to affect lipid metabolism for at least the last three months, or unwilling to stop taking any supplement at least two weeks before the study. A total of 125 participants completed the trial; however, gene expression analysis was performed on a subsample of 48 participants.

4.3.2 Ethics

The protocol was reviewed and approved by an institutional ethics board at each participating clinical site. The trial was registered at clinicaltrials.gov as NCT02029833. Written informed consent was obtained from all participants at the beginning of the study. Genetic consent was obtained from all participants included in genetic analyses.

4.3.3 Study diet

This study consisted of three treatment periods during which participants consumed a controlled iso-caloric, full-feeding diet with a fixed macronutrient composition of 35% fat, 50% carbohydrate, and 15% protein of total energy. The three phases were identical except for the type of treatment oil provided **Table 4.1**. The Control oil blend was prepared using commercially available ghee/butter oil (36.0%, Verka, New Delhi, Delhi, India), safflower oil (34.9%, eSutras, Illinois, Chicago, USA), coconut oil (16.0%, eSutras, Illinois, Chicago, USA), and flaxseed oil (13.1%, Shape Foods, MB, Canada).

Treatment phases extended for six weeks and were separated by six-week washout periods, the washout peroids ranged from four to twelve-week in some cases to meet the participants' needs. The treatment oils which comprised of 20% of total energy were incorporated into smoothie beverages which were equally divided into two portions consumed at breakfast and supper. Study food and treatment shakes were prepared based on a seven-day rotating menu cycle in the metabolic kitchen of the participating sites. During weekdays, participants consumed their breakfast and morning shake in the clinical site and collected their meals and second smoothie in food cooler bags. Weekend meals and treatment shakes were delivered to participants' residence or handed out to them, upon their request, at the clinical site

on Fridays. During the washout period, participants were instructed to consume their habitual diet.

Table 4.1: Nutrient composition of diets provided across the three treatment periods[1]

Nutrient[2]	HOCO	RCO	Control
Carbohydrate	50.8	50.8	50.8
Protein	15.9	15.9	15.7
Total fat	35.3	35.3	35.2
MUFA	19.1	17.4	13.7
Oleic acid	17.9	15.5	9.1
PUFA	7.0	9.2	6.7
Linoleic acid	5.6	6.4	4.1
α-linolenic acid	0.8	2.1	1.7
SFA	6.4	6.6	12.3
Fiber (g/3000kcal)	38.5	38.5	18.1
Cholesterol (g/3000kcal)	208.5	208.5	217.2

[1]The average composition from the 7-day rotating menu, estimated at the 3000 kcal using Food Processor Nutrition Analysis Software (ESHA Research, Salem OR). Control: low-MUFA high SFA diet; HOCO: high-oleic canola oil; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; RCO: regular canola oil; SFA: saturated fatty acid.

[2]Nutrients presented as percent of total energy, unless otherwise specified.

4.3.4 Compliance

Compliance was assessed by smoothie consumption where the participants were required to consume at least 90% of the smoothies provided at each phase. Participants signed a daily checklist to verify smoothies' consumption. To maximize the compliance rate, participants were required to consume one smoothie under the supervision of a clinical coordinator for five days/week.

4.3.5 Measurement of fat mass

Dual Energy X-ray Absorptiometry (DXA) scans were performed by a trained operator using Lunar Prodigy Advance DXA (GE Healthcare, Madison, WI, USA) with the default configurations. A DXA scan was performed for all participants at initiation and termination of each dietary phase. Participants were asked to remove any metal items and heavy clothes before scanning. Regions of interests were manually adjusted using enCORE 2012 software (version 14.10.022) according to the manufacturer's instructions. Fat mass was analyzed as total fat mass, as well as four different districts including trunk, legs, android, and gynoid fat mass. The android and gynoid regions of interest were identified as per the manufacturer's instructions. The android region was defined as a portion of the abdomen that starts at the pelvis cut line and extends upward to include 20% of distance between the pelvis and neck cut lines, with the outer arms cuts as the lateral boundaries. The gynoid region is a portion of the legs with upper boundary below the pelvis cut line by 1.5 times the height of the android region, extending downward to two times the height of the android regions, with the outer leg cuts as the lateral boundaries. Further, visceral adipose tissue was assessed by the CoreScan feature in enCORE 2012 software (version 14.10.022), and used to calculate the subcutaneous adipose tissue (SCAT) by

subtracting visceral mass from android fat mass (16). Criteria used to identify the anatomical region of interest were identical across all sites.

4.3.6 Gene expression

On the last day of each dietary phase, whole blood samples, informative and accessible tissue for an exploratory analysis, were collected using PAXGene blood RNA tubes (Qiagen, Valencia, CA). All samples were stored at -80°C until further analysis at the State University of New York at Buffalo in New York, United States. RNA was isolated from whole blood samples using the PAXGene Blood RNA purification kit (Qiagen, Valencia, CA) according to manufacturer's instructions. The concentration and purity of the isolated RNA were evaluated using NanoDrop (Thermo Fisher, Wilmington, DE). Afterward, 1 µg of the isolated RNA was used to synthesize the complementary DNA (cDNA) using the iScript advanced cDNA synthesis kit (Bio-Rad, USA). Gene expression of 22 selected genes and transcription factors related to obesity was conducted by real-time quantitative PCR using Bio-Rad PrimePCR gene expression assays (Bio-Rad, USA) and the CFX Manager software (Bio-Rad, USA). Samples were analyzed in duplicate. Expression levels of *FABP4*, *PPARGC1A*, and *SLC27A1* were too low to be detected in the majority of samples and did not allow reliable data to be obtained, therefore, these genes were not retained in the analyses.

The main objective of COMIT II was to assess the effect of HOCO and RCO on body composition compared to the Control treatment. Therefore, for fold change calculation, the Control oil treatment was considered as the control treatment. Fold changes in gene expression were calculated using the $2^{\wedge-\Delta\Delta Ct}$ method (17), i.e. 2^(mean ΔCt from the HOCO or RCO oil treatments - mean ΔCt from the Control oil treatment). ΔCt were calculated as Ct values of a

selected target gene - Ct values of the internal control gene β-actin. Ct values used in the formula consisted of the mean of the duplicate individual Ct values.

4.3.7 Statistical analyses

Statistical analyses were performed using SAS 9.4 (SAS, Cary, NC) based on a per protocol approach. Normality was assessed using the Shapiro–Wilk test, and non-normally distributed variables were log-transformed before analysis. The results are expressed as least-squares means ± SEMs unless otherwise specified. In this exploratory analysis with a small sample size, statistical significance was set at *P*-value < 0.01 in an attempt to reduce the risk of reporting false positive effects of fatty acid modification on gene expression level. The effect of MUFA on body composition and gene expression was assessed using PROC MIXED with repeated-measure procedure with treatment as a fixed effect and subject as a repeated factor. Random effects were treatment sequence, clinical site, and participants. Pre-specified potential confounders such as age, and sex were investigated in all models. Treatment effects on endpoint-to-baseline changes in body composition of each dietary phase were assessed using Tukey-Kramer post-hoc adjustment. Pearson correlation was used to assess the relationship between relative expression level of the selected genes and treatment-to-control changes in fat mass and body weight.

4.4 Results

Due to insufficient/ poor quality RNA in samples of four participants and large change in body weight at any dietary period (endpoint-baseline >5%) of 2 participants, the final number of

participants included in this study was 42 participants, whose characteristics are presented in **Table 4.2**.

Table 4.2: Characteristics of participants at the baseline of dietary intervention[1]

Characteristic	Total (n=42)	Female (n=21)	Male (n=21)	*P*[2]
Age (years)	43.6 ± 14.0	46.4 ± 3.0	40.7 ± 3.0	0.1893
Systolic BP (mmHg)	113.7 ± 11.6	112.6 ± 2.6	114.7 ± 2.6	0.5604
Diastolic BP (mmHg)	73.5 ± 11.0	72.9 ± 2.4	74.1 ± 2.4	0.7297
Total cholesterol (mmol/L)	5.06 ± 0.86	5.14 ± 0.19	4.98 ± 0.19	0.5492
Triglycerides (mmol/L)	1.43 ± 0.68	1.29 ± 0.15	1.58 ± 0.15	0.1704
HDL-cholesterol (mmol/L)	1.39 ± 0.36	1.51 ± 0.07	1.26 ± 0.07	0.0199
LDL-cholesterol (mmol/L)	3.02 ± 0.66	3.04 ± 0.15	3.00 ± 0.15	0.8477
Glucose (mmol/L)	5.16 ± 0.36	5.13 ± 0.08	5.18 ± 0.08	0.6715
Insulin (pmol/L)	85.78 ± 56.33	84.11 ± 12.44	87.45 ± 12.44	0.8503
Waist circumference (cm)	101.8 ± 11.5	99.5 ± 2.5	104.1 ± 2.5	0.1951
Body weight (kg)	88.6 ± 19.5	81.0 ± 3.9	96.3 ± 3.9	0.0091
BMI (kg/m^2)	30.27 ± 5.25	30.66 ± 1.16	29.88 ± 1.16	0.6350
VAT mass (g)	1213 ± 666	987 ± 138	1439 ± 138	0.0258
SCAT mass (g)	2045 ± 931	2270 ± 199	1820 ± 199	0.1181
Legs fat mass (g)	11927 ± 4478	13753 ± 901	10102 ± 901	0.0066
Trunk fat mass (g)	17807 ± 6604	17741 ± 1459	17874 ± 1459	0.9487
Android fat mass (g)	3258 ± 1278	3257 ± 282	3259 ± 282	0.9963
Gynoid fat mass (g)	5566 ± 2056	6261 ± 427	4871 ± 427	0.0266
Total fat mass (g)	33789 ± 11107	35664 ± 241	31913 ± 241	0.2792
Total lean mass (g)	52026 ± 12195	42928±1766	61125±1766	<0.0001

[1]Values are means ± SEMs unless otherwise specified. BP: blood pressure; BMI: body mass index; HDL: high-density lipoprotein; LDL: low-density lipoprotein; SCAT: subcutaneous adipose tissue; VAT: visceral adipose tissue.

[2]SAS PROC MIXED procedure used to assess sex differences, $P<0.05$ was considered significant. SAS PROC MEANS used to determine the mean characteristics of the overall population.

No significant treatment effect was observed on changes in body weight or body composition **Table 4.3**. **Table 4.4** shows significant treatment effect for the relative expression of carnitine palmityl transferase-1B (*CPT1B*; *p*-treatment= 0.0091); specifically, *CPT1B* expression level after RCO vs Control treatment elevated by 17% and was significantly higher than the difference after HOCO vs Control (3% reduction). There were no significant treatment effects on relative expression levels of *ACOX1*, *CD36*, *CPT1A*, *DGAT1*, *FTO*, *LEP*, *LIPC*, *LIPE*, *LPL*, perilipin-1 (*PLIN1*), *PPARδ*, *PPARGC1B*, *PRKAA2*, *RETN*, stearoyl-CoA desaturase-1 (*SCD1*), *SCD5*, *SREBF1*, and *UCP2*. **Table 4.5** shows the correlations by treatment between the relative expression of selected genes and total and regional fat mass. Following RCO consumption, negative moderate correlation (r^2= -0.518, *p*=0.002) was observed between SCAT mass and the *PLIN1* expression. Also, negative moderate correlation (r^2= -0.40, *p*=0.008) was found upon consumption of RCO between *SCD1* expression level and gynoid fat mass. Expression levels of other genes did not correlate with fat mass changes following RCO. No significant correlation was observed between expression level of any gene and fat mass following HOCO consumption.

Table 4.3: Changes in body composition in response to dietary intervention[1]

Variable	HOCO	RCO	Control	P^2
VAT (g)	-51.7 ± 24.7	-52.2 ± 24.7	-60.7 ± 24.7	0.9582
SCAT (g)	-22.1 ± 31.0	-61.2 ± 30.9	-36.9 ± 31.1	0.1169
Leg fat (g)	-233.5 ± 65.9	-110.2 ± 65.9	-158.5 ± 65.9	0.3954
Trunk fat (g)	-466.2 ± 149.0	-460.8 ± 148.9	-422.5 ± 149.1	0.9642
Android fat (g)	-70.1 ± 41.0	-112.6 ± 40.9	-107.7 ± 41.0	0.5669
Gynoid fat (g)	-123.7 ± 36.0	-92.7 ± 36.0	-115.9 ± 36.0	0.7497
Total fat (g)	-701.9 ± 166.2	-627.3 ± 166.2	-591.3 ± 166.2	0.8734
Body weight (g)	-1045.2 ± 246.8	-897.3 ± 246.8	-875.8 ± 246.8	0.8432

[1]All data represent the endpoint measures and are presented as least-squares means ± SEMs (n= 42). HOCO: high-oleic acid canola oil and RCO: regular canola oil; SCAT: subcutaneous adipose tissue; VAT: visceral adipose tissue.

[2]SAS PROC MIXED with repeated measure procedure used to assess the effect of MUFA consumption on expression levels of the candidate genes, with treatment as a fixed effect and subject as a repeated factor. Random effects were treatment sequence, clinical site, and participants. $P<0.05$ was considered significant.

Table 4.4: Mean fold change in expression levels of obesity-related genes and transcription factors compared with the Control oil[1]

Gene	Full name	Fold change vs. Control[2]		
		HOCO	RCO	*P*[3]
ACOX1	Acyl-CoA oxidase-1	1.06 ± 0.06	1.02 ± 0.06	0.3149
CD36	Cluster of differentiation 36	1.14 ± 0.08	1.10 ± 0.08	0.5076
CPT1A	Carnitine palmityl transferase-1A	1.05 ± 0.08	1.08 ± 0.08	0.5176
CPT1B	Carnitine palmityl transferase-1B	0.97 ± 0.09	1.17 ± 0.09	**0.0091**
DGAT1	Diacylglycerol O-acyltransferase	1.15 ± 0.14	1.14 ± 0.14	0.7665
FTO	Fat mass and obesity associated gene	0.95 ± 0.06	0.95 ± 0.06	0.9837
LEP	Leptin	0.02 ± 0.00	0.02 ± 0.00	0.1884
LIPC	Hepatic lipase	1.41 ± 0.35	1.39 ± 0.35	0.6228
LIPE	Hormone-sensitive lipase	1.12 ± 0.12	1.30 ± 0.12	0.2432
LPL	Lipoprotein lipase	1.16 ± 0.20	1.40 ± 0.20	0.4653
PLIN1	Perilipin-1	1.20 ± 0.26	0.96 ± 0.25	0.6339
PPARδ	Peroxisome proliferator-activated receptor delta	1.22 ± 0.24	1.22 ± 0.24	0.6191
PPARGC1B	Peroxisome proliferator-activated receptor gamma, coactivator 1 beta	1.07 ± 0.14	1.49 ± 0.14	0.1396
PRKAA2	Protein kinase, AMP activated, alpha catalytic subunit-2	1.77 ± 0.59	1.55 ± 0.59	0.1072
RETN	Resistin	1.01 ± 0.05	1.01 ± 0.05	0.9069
SCD	Stearoyl-CoA desaturase	1.25 ± 0.36	1.24 ± 0.36	0.4363
SCD5	Stearoyl-CoA desaturase-5	1.31± 0.35	1.25 ± 0.35	0.9464
SREBF1	Steroyl response element binding protein-1	1.79 ± 0.78	1.76 ± 0.78	0.3931
UCP2	Uncoupling protein-2	1.20 ± 0.12	1.15 ± 0.12	0.6120

[1]All data are represented as least-squares means ± SEMs (n= 42). Ct: cycle threshold; HOCO: high-oleic acid canola oil; RCO: regular canola oil.

[2]Fold change in gene expression calculated using the 2^ΔΔCt method, i.e. 2^(mean ΔCt from the HOCO or RCO oil treatments - mean ΔCt from the Control oil treatment). ΔCt were calculated as Ct values of a selected target gene - Ct values of the internal control gene β-actin. Ct values used in the formula consisted of the mean of the duplicate individual Ct values.

[3]*P* value of the main treatment effect. SAS PROC MIXED with repeated measure procedure used to assess the effect of MUFA consumption on the fold expression levels of the candidate genes, with treatment as a fixed effect and subject as a repeated factor. Random effects were treatment sequence, clinical site, and participants. $P<0.05$ was considered significant.

Table 4.5: Correlations between total and regional fat mass and relative expression of selected obesity-related genes and transcription factors[1]

	VAT		SCAT		Android fat		Gynoid fat		Total fat	
	HOCO	RCO	HOCO	RCO	HOCO	RCO	HOCO	RCO	HOCO	RCO
ACOX1[2]	0.136, 0.389	0.053, 0.737	0.027, 0.865	0.045, 0.777	0.108, 0.495	0.079, 0.618	0.180, 0.254	0.143, 0.365	0.184, 0.245	0.086, 0.588
CD36	0.051, 0.749	0.057, 0.719	0.087, 0.585	-0.040, 0.799	0.091, 0.567	0.015, 0.927	-0.008, 0.961	-0.018, 0.908	0.070, 0.658	-0.019, 0.904
CPT1B	0.120, 0.449	0.074, 0.643	0.015, 0.925	-0.071, 0.653	0.089, 0.573	0.003, 0.983	-0.242, 0.122	0.001, 0.997	-0.103, 0.516	-0.011, 0.943
CPT1A	0.226, 0.151	0.103, 0.518	-0.031, 0.847	0.008, 0.961	0.129, 0.414	0.090, 0.572	0.010, 0.949	0.060, 0.704	0.106, 0.505	0.070, 0.660
DGAT1	0.169, 0.284	0.171, 0.279	0.193, 0.220	0.091, 0.568	0.240, 0.126	0.211, 0.179	0.231, 0.141	0.095, 0.550	0.286, 0.066	0.101, 0.524
FTO	-0.171, 0.280	-0.064, 0.688	-0.125, 0.429	0.213, 0.176	-0.196, 0.213	0.117, 0.462	-0.112, 0.479	0.174, 0.272	-0.207, 0.189	0.065, 0.681
LEP	-0.110, 0.593	-0.091, 0.607	-0.022, 0.915	0.079, 0.658	-0.085, 0.678	-0.007, 0.970	0.047, 0.821	-0.050, 0.777	-0.043, 0.836	-0.099, 0.579
LIPC	-0.288, 0.065	-0.114, 0.472	0.161, 0.308	-0.293, 0.060	-0.085, 0.595	-0.325, 0.036	-0.082, 0.604	-0.335, 0.030	-0.067, 0.675	-0.325, 0.036
LIPE	-0.039, 0.806	0.180, 0.254	-0.092, 0.562	-0.124, 0.433	-0.087, 0.585	0.048, 0.762	-0.168, 0.288	0.019, 0.906	-0.131, 0.407	0.039, 0.806
LPL	0.074, 0.644	-0.030, 0.852	0.030, 0.851	0.113, 0.477	0.069, 0.666	0.065, 0.682	-0.156, 0.325	-0.040, 0.800	0.062, 0.695	-0.028, 0.863
PLIN1	-0.294, 0.122	0.108, 0.551	0.084, 0.664	**-0.518, 0.002**	-0.150, 0.438	-0.310, 0.079	-0.099, 0.611	-0.353, 0.044	-0.118, 0.541	-0.268, 0.132

Table 4.5: Continued

	VAT		SCAT		Android		Gynoid		Total fat	
	HOCO	RCO	HOCO	RCO	HOCO	RCO	HOCO	RCO	HOCO	RCO
PPARδ	-0.339, 0.028	-0.164, 0.298	-0.010, 0.948	-0.116, 0.466	-0.232, 0.140	-0.226, 0.151	-0.208, 0.186	-0.228, 0.147	-0.174, 0.270	-0.266, 0.089
PPARGC1B	-0.091, 0.567	0.032, 0.842	0.144, 0.363	-0.060, 0.707	0.035, 0.827	-0.021, 0.893	-0.049, 0.759	-0.026, 0.869	0.003, 0.985	-0.088, 0.579
PRKAA2	-0.181, 0.253	-0.031, 0.843	0.089, 0.574	-0.152, 0.335	-0.061, 0.702	-0.146, 0.355	-0.103, 0.517	-0.237, 0.130	-0.007, 0.967	-0.151, 0.341
RETN	-0.025, 0.873	-0.062, 0.700	0.113, 0.477	-0.017, 0.916	0.058, 0.717	-0.064, 0.691	-0.025, 0.877	-0.184, 0.249	0.072, 0.649	-0.125, 0.437
SCD	-0.328, 0.034	-0.110, 0.487	0.111, 0.486	-0.295, 0.058	-0.144, 0.362	-0.324, 0.037	-0.075, 0.635	**-0.402, 0.008**	-0.068, 0.668	-0.282, 0.071
SCD5	-0.264, 0.092	0.028, 0.860	-0.037, 0.815	-0.083, 0.601	-0.199, 0.206	-0.043, 0.788	-0.112, 0.481	-0.009, 0.957	-0.194, 0.218	-0.021, 0.893
SREBF1	-0.302, 0.052	-0.097, 0.540	0.086, 0.589	-0.165, 0.296	-0.144, 0.364	-0.210, 0.181	-0.165, 0.297	-0.156, 0.325	-0.163, 0.303	-0.161, 0.309
UCP2	-0.157, 0.322	0.066, 0.680	-0.084, 0.596	0.061, 0.703	-0.159, 0.313	0.102, 0.522	-0.157, 0.321	0.050, 0.752	-0.144, 0.361	0.105, 0.508

[1]All data are represented as correlation coefficient and *p*-value (n= 42). Pearson correlation calculated between fold change in gene expression and changes in fat mass from treatment phase-to-Control phase. Fold change in gene expression calculated using the 2^ΔΔCt method, i.e. 2^(mean ΔCt from the HOCO or RCO oil treatments - mean ΔCt from the Control). ΔCt were calculated as Ct values of a selected target gene - Ct values of the internal control gene β-actin. Ct values used in the formula consisted of the mean of the duplicate individual Ct values. Change in fat mass was calculated by subtracting the fat mass of Control phase from fat mass following HOCO or RCO. $P<0.01$ was considered significant.

[2]*ACOX1*: acyl-CoA oxidase-1; *CD36*: cluster of differentiation 36; *CPT1A*: carnitine palmityl transferase-1A; *CPT1B*: carnitine palmityl transferase-1B; Ct: cycle threshold; *DGAT1*: diacylglycerol O-acyltransferase; *FTO*: fat mass and obesity associated gene; HOCO: high-oleic acid canola oil; *LEP*: leptin; *LIPC*: hepatic lipase; *LIPE*: hormone-sensitive lipase; *LPL*: lipoprotein lipase; *PLIN1*: perilipin-1; *PPARδ*: peroxisome proliferator-activated receptor delta; *PPARGC1B*: peroxisome proliferator-activated receptor gamma, coactivator 1 beta; *PRKAA2*: protein kinase, AMP activated, alpha catalytic subunit; RCO: regular canola oil; *RETN*: resistin; SCAT: subcutaneous adipose tissue *SCD*: stearoyl-CoA desaturase; *SCD5*; stearoyl-CoA desaturase-5; *SREBF1*: steroyl response element binding protein-1; *UCP2*: uncoupling protein-2; VAT: visceral adipose tissue.

4.5 Discussion

This study found a significant modulatory effect of MUFA on the relative expression of *CPT1B* in a controlled full-feeding condition. This finding in addition to the detected correlations between *PLIN1* and *SCD1* expression levels and regional fat mass might help illuminate an underlying mechanism of the beneficial effects of MUFA consumption on energy balance that has been previously conveyed in the literature.

CPT1 is the rate-limiting enzyme that regulates the capacity for mitochondrial long-chain fatty acid β-oxidation, therefore, an increased expression of *CPT1* may stimulate oxidation level (18, 19). *CPT1A* is expressed in several tissues including liver, whereas *CPT1B* is expressed mainly in skeletal muscle, heart, and brown adipose tissue (19). Here, MUFA consumption for six weeks influenced the expression of *CPT1B,* but not *CPT1A*. New avenues of treatment for obesity and type II diabetes have targeted the *CPT1* gene (19). The abundance of *CPT1* may significantly contribute to the fatty acid oxidation capacity (20), and has been previously reported to be influenced by dietary fatty acid consumption in humans (21, 22). One study in human adipose stem cells treated with fatty acids for 12 days found no significant effect of oleic acid on expression of several genes that regulate mitochondrial function, including *CPT1B* (23). An animal study found that a long-chain MUFA-rich diet elevated expression levels of *Cpt1A* by 180% in mesenteric white adipose tissue and by 120% in subcutaneous adipose tissue, as compared to a control diet (11). Our results add another dimension to the available evidence in that we found that the abundance of *CPT1B* significantly influenced by the dietary level of MUFA in this controlled iso-energetic diet design experiment.

This finding might explain the evidence from clinical trials, which indicates the superiority of MUFA-rich diet in improving fat oxidation and resting expenditure compared to

other fatty acids (2, 4-7). However, we failed to detect a concomitant reduction in fat mass in response to dietary intervention, which might be attributed to several possible reasons. First, a low abundance of *CPT1* exists in white adipose tissue (19) which therefore, might not be sufficient to induce fat loss. Second, although modulating *CPT1B* gene abundance by MUFA may be a pivotal component of the adaptive capacity of skeletal muscle to match the elevated consumption of these fatty acids by using them as predominant fuel source (20), a prolonged period might be required to achieve an effect on fat mass in humans. Further, DXA cannot distinguish subcutaneous fat from intramuscular fat, which limits our ability to detect the possible local influence of the elevated expression of *CPT1B*.

MUFA consumption modulated the correlation between *PLIN1* and *SCD1* expression levels and body fat mass. *SCD* encodes the rate-limiting enzyme that catalyzes the conversion of SFA to MUFA (24, 25), and its expression is regulated by diet and transcription factors (26). Although *SCD* is implicated in obesity development, mechanisms by which SCD or its MUFA product might modulate the metabolism and obesity risk are yet to be elucidated (24). Present evidence shows conflicting results regarding the effect of *SCD* expression on obesity and its metabolic complication (26, 27). However, *SCD* might improve membrane fluidity by optimizing the SFA:MUFA ratio which could reduce the risk of several pathological status including obesity (26, 28).

PLIN1 encodes a lipid-droplet associated protein that controls the efficiency and capacity of lipolysis in adipocyte (29). Suppression of *PLIN1* increases lipolysis and prevents diet-induced obesity in mice (29, 30). However, overexpression of *PLIN1* resulted in a reduction in expression levels of lipid synthesis genes and an elevation in the expression of β-oxidation and thermogenesis genes (31). A previous study found no effect of dietary fatty acid composition on

PLIN1 expression level in human skeletal muscle (32). The link between obesity and *PLIN1* expression level has been scarcely studied in human and the results are inconsistent. Kern et al. reported a positive correlation between *PLIN1* expression level and percent body fat (30). In contrast, other studies have shown a lower *PLIN1* relative abundance in adipose tissue from obese compared to lean subjects (29, 33). The observed negative correlations between *PLIN1* and *SCD1* expression levels and regional fat mass following RCO consumption might point to these genes as promising targets in obesity-combating dietary intervention.

Small sample size might have limited our ability to detect significant differences in expression levels of the measured genes and transcription factors, especially those with expression levels of ≥50% greater than the Control treatment. Also, from our trial, we cannot explain the reduction in *CPT1B* relative expression level or the lack of correlations between *PLIN1* and *SCD1* expression levels and body fat mass following HOCO treatment compared to RCO. Further, measurements taken from mRNA and protein levels are complementary and both are necessary for a complete understanding of a biological mechanism, where higher expression levels do not necessarily associate with higher level or activity of the produced enzyme/protein (34). Assessing enzyme/protein activity is warranted in future studies to obtain a broader picture regarding the effect of different levels of MUFA on these pathways. Further, the association between genes and obesity can be modifies by genetic polymorphisms. Polymorphisms within *CPT1* (35), *SCD1* (36), and *PLIN1* (37) were associated with obesity, therefore, assessing the association of polymorphisms within these genes and dietary fatty acid composition on body fat mass is highly encouraged.

In summary, the current study is one of the first evaluating the influence of fatty acid composition on gene expression in a controlled condition in humans, and may begin to elucidate

the biological pathways by which dietary MUFA can induce favorable effect on energy balance. Here, we add to the present evidence that supports the role of MUFA consumption in augmenting energy expenditure and fat oxidation (2, 5, 6), by identifying *CPT1B* as a target gene for dietary MUFA in humans.

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4.6 References

1. Gillingham LG, Robinson KS, Jones PJ. Effect of high-oleic canola and flaxseed oils on energy expenditure and body composition in hypercholesterolemic subjects. Metabolism 2012;61(11):1598-605. doi: 10.1016/j.metabol.2012.04.016.

2. Krishnan S, Cooper JA. Effect of dietary fatty acid composition on substrate utilization and body weight maintenance in humans. Eur J Nutr 2014;53(3):691-710. doi: 10.1007/s00394-013-0638-z.

3. Field AE, Willett WC, Lissner L, Colditz GA. Dietary fat and weight gain among women in the Nurses' Health Study. Obesity (Silver Spring) 2007;15(4):967-76. doi: 10.1038/oby.2007.616.

4. Kien CL, Bunn JY. Gender alters the effects of palmitate and oleate on fat oxidation and energy expenditure. Obesity (Silver Spring) 2008;16(1):29-33. doi: 10.1038/oby.2007.13.

5. Piers LS, Walker KZ, Stoney RM, Soares MJ, O'Dea K. The influence of the type of dietary fat on postprandial fat oxidation rates: monounsaturated (olive oil) vs saturated fat (cream). Int J Obes Relat Metab Disord 2002;26(6):814-21. doi: 10.1038/sj.ijo.0801993.

6. Kien CL, Bunn JY, Tompkins CL, Dumas JA, Crain KI, Ebenstein DB, Koves TR, Muoio DM. Substituting dietary monounsaturated fat for saturated fat is associated with increased daily physical activity and resting energy expenditure and with changes in mood. Am J Clin Nutr 2013;97(4):689-97. doi: 10.3945/ajcn.112.051730.

7. Piers LS, Walker KZ, Stoney RM, Soares MJ, O'Dea K. Substitution of saturated with monounsaturated fat in a 4-week diet affects body weight and composition of overweight and obese men. Br J Nutr 2003;90(3):717-27.

8. Liu X, Kris-Etherton PM, West SG, Lamarche B, Jenkins DJ, Fleming JA, McCrea CE, Pu S, Couture P, Connelly PW, et al. Effects of canola and high-oleic-acid canola oils on abdominal fat mass in individuals with central obesity. Obesity (Silver Spring) 2016;24(11):2261-8. doi: 10.1002/oby.21584.

9. Larter CZ, Yeh MM, Cheng J, Williams J, Brown S, dela Pena A, Bell-Anderson KS, Farrell GC. Activation of peroxisome proliferator-activated receptor alpha by dietary fish oil attenuates steatosis, but does not prevent experimental steatohepatitis because of hepatic lipoperoxide accumulation. J Gastroenterol Hepatol 2008;23(2):267-75. doi: 10.1111/j.1440-1746.2007.05157.x.

10. Muhlhausler BS, Cook-Johnson R, James M, Miljkovic D, Duthoit E, Gibson R. Opposing effects of omega-3 and omega-6 long chain polyunsaturated Fatty acids on the expression of lipogenic genes in omental and retroperitoneal adipose depots in the rat. J Nutr Metab 2010;2010. doi: 10.1155/2010/927836.

11. Yang ZH, Miyahara H, Iwasaki Y, Takeo J, Katayama M. Dietary supplementation with long-chain monounsaturated fatty acids attenuates obesity-related metabolic dysfunction and increases expression of PPAR gamma in adipose tissue in type 2 diabetic KK-Ay mice. Nutr Metab (Lond) 2013;10(1):16. doi: 10.1186/1743-7075-10-16.

12. Bueno AA, Oyama LM, de Oliveira C, Pisani LP, Ribeiro EB, Silveira VL, Oller do Nascimento CM. Effects of different fatty acids and dietary lipids on adiponectin gene expression in 3T3-L1 cells and C57BL/6J mice adipose tissue. Pflugers Arch 2008;455(4):701-9. doi: 10.1007/s00424-007-0330-3.

13. Kim J, Carlson ME, Kuchel GA, Newman JW, Watkins BA. Dietary DHA reduces downstream endocannabinoid and inflammatory gene expression and epididymal fat mass while improving aspects of glucose use in muscle in C57BL/6J mice. Int J Obes (Lond) 2016;40(1):129-37. doi: 10.1038/ijo.2015.135.

14. Reilly JM, Thompson MP. Dietary fatty acids Up-regulate the expression of UCP2 in 3T3-L1 preadipocytes. Biochem Biophys Res Commun 2000;277(3):541-5. doi: 10.1006/bbrc.2000.3705.

15. Paniagua JA, Gallego de la Sacristana A, Romero I, Vidal-Puig A, Latre JM, Sanchez E, Perez-Martinez P, Lopez-Miranda J, Perez-Jimenez F. Monounsaturated fat-rich diet prevents central body fat distribution and decreases postprandial adiponectin expression induced by a carbohydrate-rich diet in insulin-resistant subjects. Diabetes Care 2007;30(7):1717-23. doi: 10.2337/dc06-2220.

16. Miazgowski T, Krzyzanowska-Swiniarska B, Dziwura-Ogonowska J, Widecka K. The associations between cardiometabolic risk factors and visceral fat measured by a new dual-energy X-ray absorptiometry-derived method in lean healthy Caucasian women. Endocrine 2014;47(2):500-5. doi: 10.1007/s12020-014-0180-7.

17. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001;25(4):402-8. doi: 10.1006/meth.2001.1262.

18. Ulven SM, Myhrstad MC, Holven KB. Marine n-3 fatty acids and gene expression in peripheral blood mononuclear cells. Edtion ed. Curr Cardiovasc Risk Rep. Boston, 2014.

19. Warfel JD, Vandanmagsar B, Dubuisson OS, Hodgeson SM, Elks CM, Ravussin E, Mynatt RL. Examination of carnitine palmitoyltransferase 1 abundance in white adipose tissue: implications in obesity research. Am J Physiol Regul Integr Comp Physiol 2017;312(5):R816-r20. doi: 10.1152/ajpregu.00520.2016.

20. Cameron-Smith D, Burke LM, Angus DJ, Tunstall RJ, Cox GR, Bonen A, Hawley JA, Hargreaves M. A short-term, high-fat diet up-regulates lipid metabolism and gene expression in human skeletal muscle. Am J Clin Nutr 2003;77(2):313-8.

21. Myhrstad MC, Narverud I, Telle-Hansen VH, Karhu T, Lund DB, Herzig KH, Makinen M, Halvorsen B, Retterstol K, Kirkhus B, et al. Effect of the fat composition of a single high-fat

meal on inflammatory markers in healthy young women. Br J Nutr 2011;106(12):1826-35. doi: 10.1017/s0007114511002510.

22. Yoshino J, Smith GI, Kelly SC, Julliand S, Reeds DN, Mittendorfer B. Effect of dietary n-3 PUFA supplementation on the muscle transcriptome in older adults. Edtion ed. Physiol Rep. Hoboken, 2016.

23. Fleckenstein-Elsen M, Dinnies D, Jelenik T, Roden M, Romacho T, Eckel J. Eicosapentaenoic acid and arachidonic acid differentially regulate adipogenesis, acquisition of a brite phenotype and mitochondrial function in primary human adipocytes. Mol Nutr Food Res 2016;60(9):2065-75. doi: 10.1002/mnfr.201500892.

24. Liu J, Cinar R, Xiong K, Godlewski G, Jourdan T, Lin Y, Ntambi JM, Kunos G. Monounsaturated fatty acids generated via stearoyl CoA desaturase-1 are endogenous inhibitors of fatty acid amide hydrolase. Edtion ed. Proc Natl Acad Sci U S A, 2013:18832-7.

25. Hu CC, Qing K, Chen Y. Diet-induced changes in stearoyl-CoA desaturase 1 expression in obesity-prone and -resistant mice. Obes Res 2004;12(8):1264-70. doi: 10.1038/oby.2004.160.

26. Popeijus HE, Saris WH, Mensink RP. Role of stearoyl-CoA desaturases in obesity and the metabolic syndrome. Int J Obes (Lond) 2008;32(7):1076-82. doi: 10.1038/ijo.2008.55.

27. Matsui H, Yokoyama T, Sekiguchi K, Iijima D, Sunaga H, Maniwa M, Ueno M, Iso T, Arai M, Kurabayashi M. Stearoyl-CoA Desaturase-1 (SCD1) augments saturated fatty acid-induced lipid accumulation and inhibits apoptosis in cardiac myocytes. Edtion ed. In: Stadler K, ed. PLoS One. San Francisco, USA, 2012.

28. Alarcón G, Roco J, Medina A, Van Nieuwenhove C, Medina M, Jerez S. Stearoyl-CoA desaturase indexes and n-6/n-3 fatty acids ratio as biomarkers of cardiometabolic risk factors in normal-weight rabbits fed high fat diets. Edtion ed. J Biomed Sci. London, 2016.

29. Ray H, Pinteur C, Frering V, Beylot M, Large V. Depot-specific differences in perilipin and hormone-sensitive lipase expression in lean and obese. Lipids Health Dis 2009;8:58. doi: 10.1186/1476-511x-8-58.

30. Kern PA, Di Gregorio G, Lu T, Rassouli N, Ranganathan G. Perilipin expression in human adipose tissue is elevated with obesity. J Clin Endocrinol Metab 2004;89(3):1352-8. doi: 10.1210/jc.2003-031388.

31. Moreno-Navarrete JM, Ortega F, Serrano M, Rodriguez-Hermosa JI, Ricart W, Mingrone G, Fernandez-Real JM. CIDEC/FSP27 and PLIN1 gene expression run in parallel to mitochondrial genes in human adipose tissue, both increasing after weight loss. Int J Obes (Lond) 2014;38(6):865-72. doi: 10.1038/ijo.2013.171.

32. Gjelstad IM, Haugen F, Gulseth HL, Norheim F, Jans A, Bakke SS, Raastad T, Tjonna AE, Wisloff U, Blaak EE, et al. Expression of perilipins in human skeletal muscle in vitro and in vivo in relation to diet, exercise and energy balance. Arch Physiol Biochem 2012;118(1):22-30. doi: 10.3109/13813455.2011.630009.

33. Wang Y, Sullivan S, Trujillo M, Lee MJ, Schneider SH, Brolin RE, Kang YH, Werber Y, Greenberg AS, Fried SK. Perilipin expression in human adipose tissues: effects of severe obesity, gender, and depot. Obes Res 2003;11(8):930-6. doi: 10.1038/oby.2003.128.

34. Maier T, Güell M, Serrano L. Correlation of mRNA and protein in complex biological samples. FEBS Letters 2009;583(24):3966-73. doi: https://doi.org/10.1016/j.febslet.2009.10.036.

35. Robitaille J, Houde A, Lemieux S, Perusse L, Gaudet D, Vohl MC. Variants within the muscle and liver isoforms of the carnitine palmitoyltransferase I (CPT1) gene interact with fat intake to modulate indices of obesity in French-Canadians. J Mol Med (Berl) 2007;85(2):129-37. doi: 10.1007/s00109-006-0116-7.

36. Warensjo E, Ingelsson E, Lundmark P, Lannfelt L, Syvanen AC, Vessby B, Riserus U. Polymorphisms in the SCD1 gene: associations with body fat distribution and insulin sensitivity. Obesity (Silver Spring) 2007;15(7):1732-40. doi: 10.1038/oby.2007.206.

37. Konige M, Wang H, Sztalryd C. Role of adipose specific lipid droplet proteins in maintaining whole body energy homeostasis. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 2014;1842(3):393-401. doi: https://doi.org/10.1016/j.bbadis.2013.05.007.

BRIDGE TO CHAPTER V

The concomitant association between abdominal obesity and IR necessitates evaluating the effectiveness of dietary FA modification, as a previously acknowledged modulator of IR risk, in subjects with abdominal obesity. Given the evidence provided in Chapter III and Chapter IV regarding the effect of interactions between genetics and MUFA on modifying fat mass changes, Chapter V comprises a manuscript that evaluates a potential role of common variants within IR-candidate genes on the responsiveness of IR measure to different levels of dietary MUFA. Shatha Hammad participated in study coordination and data collection at RCFFN, performed the genotype analysis and data interpretation, conducted the statistical analyses, and wrote the manuscript.

CHAPTER V

MANUSCRIPT 4

This manuscript is under submission to the

Polymorphisms in *IRS1* and *FABP1* modify insulin resistance in response to fatty acid composition in non-diabetic subjects with abdominal obesity

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Running Head: Genetics and fatty acid quality modify insulin resistance

Clinical Trial Registry: The trial was registered at clinicaltrials.gov as NCT02029833.

5.1 Abstract

Background: The prevalence rates of insulin resistance (IR) and its health consequences are increasing worldwide. Emerging evidence suggests a modulatory effect of single nucleotide polymorphisms (SNPs) on IR response to dietary fatty acid (FA) composition; yet, evidence from clinical trials is missing.

Objective: We evaluated the response of IR measures to different levels of dietary FA composition, and the impact of IR-associated genetic polymorphisms on this response.

Methods: Non-diabetic adults (n = 116) with abdominal obesity were included in a randomized, controlled-feeding, double-blinded, crossover, multicentre trial. During each phase, participants consumed one of three treatment oils (20% of total fat) for six weeks, separated by a four-twelve week washout. Treatment oils included two-high monounsaturated FA (MUFA) oils, conventional canola or high-oleic acid canola, or a low-MUFA high-saturated FA (SFA) oil blend. Genotyping of nine candidate SNPs was performed using qualitative PCR System.

Results: In the sample as a whole, no differences across the three diets were observed for fasting concentrations of glucose, insulin, and fructosamine, as well as on HOMA-IR or HOMA-β. In the homozygotes of *FABP1* rs2241883-CC, but not in other genotypes, reductions in HOMA-IR (p= 0.042) and fasting insulin (trend, p= 0.06) were observed following consumption of the high-SFA diet compared to the high-MUFA diets. Reductions in insulin (p= 0.04), HOMA-β (p= 0.02) and HOMA-IR (trend, p= 0.07) in the *IRS1* rs7578326-GG homozygotes were also observed upon consumption of the high-SFA diet compared to the high-MUFA diets. Polymorphisms within *ADIPOQ*, *ADRB2*, *FTO*, *PLIN1*, *PPARγ*, and *TCF7L2* genes failed to modulate the effect of MUFA consumption on glucose homeostasis and IR measures.

Conclusion: Dietary FA composition modified IR measures in the carriers of either the *FABP1* rs2241883-CC or *IRS1* rs7578326-GG genotype. These results may contribute to developing an effective genotype-based dietary recommendation to reduce IR incidence and associated complications.

5.2 Introduction

Insulin resistance (IR) increases the risk of type 2 diabetes and several metabolic abnormalities (1, 2). Growing evidence underscores the role of dietary fatty acid (FA) composition in the development of IR (3). The quality of dietary fat potentially impacts the efficiency of action of insulin signaling pathways by modifying cellular membrane fluidity and/or increasing intramuscular lipid content (4, 5). Also, dietary fat quality may be involved in IR risk by influencing glucose-stimulated insulin secretion (3). Dietary FAs with greater degrees of saturation and/or chain length were suggested to negatively affect the secretion and sensitivity of insulin (5, 6). In contrast, monounsaturated FA (MUFA) consumption has been reported to ameliorate IR compared to a saturated FA (SFA)-rich diet in non-diabetic subjects (3, 7, 8) and carbohydrate-rich diet in diabetic patients (9). Yet, the evidence is inconsistent (10-13). Genetic predisposition may contribute to the responsiveness of IR to dietary FA, and therefore, may explain the discrepancies in the available evidence.

Many polymorphisms have been identified to be associated with IR risk, independent of insulin secretion, with an estimated heritability of 60% in familial and twin studies (14-16). Among the IR-associated genetic variants, those within the insulin receptor substrate-1 (*IRS1*) gene have garnered more attention. *IRS1*, encoding a major signaling adaptor protein for insulin, is expressed in insulin-sensitive tissues and plays a pivotal role in insulin-stimulated signaling pathways (17, 18). Reductions in the activity and/or expression of *IRS1* could contribute to IR and type 2 diabetes (19-21). Another candidate gene for IR is *FABP1* which encodes the liver fatty acid binding protein and serves as a key regulator of lipid metabolism (22). Polymorphisms that alter the functionality of *FABP1* may modify hepatic triglyceride accumulation and FA flux

to the liver and, thus, may influence hepatic IR (16, 23). As such, an emerging evidence suggests a role of genetics in explaining the action of FA composition in modulating IR (24, 25).

Given the increasing prevalence of IR and its metabolic and health consequences worldwide, establishing effective personalized prevention and treatment strategies for IR and its-associated metabolic abnormalities requires investigation of the interactions between dietary factors and common genetic variants. Therefore, we aimed to investigate the effect of polymorphisms within IR-related genes on insulin sensitivity responses to dietary FA composition in subjects with abdominal obesity.

5.3 Methods

5.3.1 Study design and population

This study was part of the Canola Oil Multi-center Intervention Trial II (COMIT II); a randomized, controlled, double-blinded, crossover study aimed to evaluate the effects of MUFA consumption on body composition and cardiovascular disease-related metabolic responses in individuals with abdominal obesity. Recruitment was conducted at four centers including: the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN) at the University of Manitoba in Winnipeg, the Canadian Centre for Agri-Food Research in Health and Medicine (CCARM) at St. Boniface Hospital Albrechtsen Research Centre in Winnipeg, the Institute of Nutrition and Functional Foods (INAF) at Laval University in Québec City, as well as the Department of Nutritional Sciences, The Pennsylvania State University in University Park. Additionally, St. Michael's Hospital in Toronto participated in sample analyses.

Participants aged 20-65 years were included in the trial if they had abdominal obesity as identified with waist circumference of greater than 94 cm for men and 80 cm for women, in

addition to at least one of the following metabolic syndrome parameters as secondary inclusion criteria: fasting blood glucose of ≥ 5.6 mmol/L (according to the American Diabetes Association definition for pre-diabetes), TG ≥1.7 mmol/L, HDL-C <1 mmol/L (men) or <1.3 mmol/L (women), and blood pressure ≥130 mmHg (systolic) and/or ≥85 mmHg (diastolic). Individuals were excluded if they had kidney, diabetes, liver, or unstable thyroid disease. Current smokers, pregnant and lactating women, individuals consuming more than two alcoholic beverages per day, individuals taking medication known to affect lipid metabolism for at least the last three months, or individuals who were unwilling to stop taking any supplements for at least two-weeks before the study were not eligible to participate.

5.3.2 Statement of ethics

Written informed consent was obtained from all participants prior to enrollment. The protocol was reviewed and approved by institutional ethics boards of the participating clinical sites. The trial was registered at clinicaltrials.gov as NCT02029833.

5.3.3 Dietary intervention

This study consisted of three six-week treatment periods separated by six-week washout periods, the washout peroids ranged from four to twelve-week in some cases to meet the participants needs. During each treatment phase, participants consumed a controlled iso-caloric, full-feeding diet containing 35% fat, 50% carbohydrate, and 15% protein of total energy, as well as ~208 mg/3000 kcal/d cholesterol and ~38 g/3000 kcal/d fiber. All three phases were identical except for the type of treatment oil provided. All meals were prepared based on a seven-day rotating menu cycle in the metabolic kitchen of the participating sites. During the washout periods, participants were instructed to consume their habitual diets. To eliminate the effect of physical activity on IR, participants were requested to maintain their usual level of planned and

structured physical activity during the study. Physical activity changes were monitored by a weekly questionnaire.

Treatment oils consisted of 20% of total energy and were incorporated into two equal portions of smoothie beverages consumed at breakfast and supper. Treatment oils included: 1) regular canola oil (RCO; Canola Harvest Canola Oil, Richardson International, MB, Canada) consisted of 6.6% SFA, 65.3% MUFA, 19.6% n-6 PUFA, 8.5% α-linolenic acid, 2) high-oleic acid canola oil (HOCO; Canola Harvest Canola Oil, Richardson International, MB, Canada) consisted of 6.7% SFA, 75.9% MUFA, 14.8% n-6 PUFA, 2.6% α-linolenic acid, and 3) a high-SFA low-MUFA control oil consisted of 22.1% long-chain SFA, 18.1% medium-chain FA (MCFA), 22.0% MUFA, 29.6% n-6 PUFA, 8.2% n-3 PUFA α-linolenic acid. The high-SFA oil blend was prepared using 34.9% safflower oil (eSutras, Illinois, Chicago, USA), 36.0% ghee/butter oil (Verka, New Delhi, Delhi, India), 16.0% coconut oil (eSutras, Illinois, Chicago, USA), and 13.1% flaxseed oil (Shape Foods, MB, Canada). Compliance was assessed by the consumption of smoothies; 90% was the required target for smoothie consumption at each phase. Participants signed a daily checklist to verify smoothies' consumption. To ensure optimal compliance, participants were required to consume one smoothie each day under a supervision of a clinical coordinator.

5.3.4 Biochemical measurements

On the first two and last two days of each treatment phase, 12-hr fasting blood samples were collected, processed, and stored at −80°C until further analyzed. Frozen samples were shipped to St. Michael's Hospital (Toronto, ON, Canada) for analysis. Serum insulin and fructosamine were measured with the Roche/Hitachi cobas-e immunoassay analyzer and electrochemiluminescence immunoassay kits (Roche Diagnostics, Laval, QC, Canada). Serum

glucose was determined using cobas® enzymatic reagents on Roche/Hitachi c501e automated clinical chemistry analyzers (Roche Diagnostics, Laval, QC, Canada). The average of the last two days was calculated and used for endpoint-to-endpoint comparison in statistical analyses.

Homeostasis model assessment (HOMA)-IR and HOMA-beta cell function (β) indices were calculated using the average of the last two days via following formulas (26):

$$\mathrm{HOMA-IR} = \frac{\text{Fasting insulin } \left(\frac{\mu\mathrm{IU}}{\mathrm{ml}}\right) \times \text{Fasting glucose } \left(\frac{\mathrm{mg}}{\mathrm{dl}}\right)}{22.5} \tag{5.1}$$

$$\mathrm{HOMA-\beta} = \frac{\text{Fasting insulin } \left(\frac{\mu\mathrm{IU}}{\mathrm{ml}}\right) \times 20}{\text{Fasting glucose (mg/dl)}} - 3.5 \tag{5.2}$$

5.3.5 Genotyping

Buffy coat samples of the first day of the first phase were used to extract the genomic DNA using Qiagen DNeasy Blood and Tissue Kit according to the manufacturer's instructions (Qiagen Sciences Inc., Toronto, ON, Canada). The concentration and purity of the extracted DNA were assessed using Thermo Scientific NanoDrop 2000 micro-volume spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). TaqMan GTXpree Master Mix with allele-specific probes (Applied Biosystems, Life Technologies Inc., Burlington, ON, Canada) was used to genotype nine candidate single-nucleotide polymorphisms (SNPs) within eight genes that have been previously associated with insulin resistance. The selected genes included adiponectin (*ADIPOQ*); adrenoceptor beta-2 (*ADRB2*); fat mass and obesity-associated gene (*FTO*); insulin

receptor substrate-1 (*IRS1*); liver fatty acid binding protein (*FABP1*); perilipin-1 (*PLIN1*); peroxisome proliferator-activated receptor gamma (*PPARγ*); transcription factor 7-like-2 (*TCF7L2*). The characteristics of the selected SNPs are presented in **Table 5.1**. Amplification and detection of DNA were conducted with the 7500 Fast Real-Time PCR System (Applied Biosystems, Life Technologies Inc, Burlington, ON, Canada) and StepOne 2.1 (Applied Biosystems, Life Technologies Inc., Burlington, ON, Canada). All samples were run in duplicate.

Table 5.1: Characteristics of the selected polymorphisms

Full name of gene	**Gene**	**SNP**	**Region**	**Allele**	**Genotype (n)**			**MAF%**
				Major/minor	MM	Mm	mm	
Adiponectin	*ADIPOQ*	rs266729	Exon/ 5' UTR	C/G	61	30	10	24.8
Adrenoceptor Beta-2	*ADRB2*	rs1042714	Exon	C/G	42	41	18	38.1
Fat mass and obesity-associated gene	*FTO*	rs9939609	Intron	T/A	44	39	18	37.1
Insulin receptor substrate-1	*IRS1*	rs2943641	Intergene	C/T	50	42	9	29.7
		rs7578326	Intron	A/G	49	45	7	29.2
Liver fatty acid binding protein	*FABP1*	rs2241883	Missense	T/C	46	45	10	32.2
Perilipin-1	*PLIN1*	rs894160	Intron	C/T	47	43	11	32.2
Peroxisome proliferator-activated receptor gamma	*PPARγ*	rs1801282	Missense	C/G	78	21	2	12.4
Transcription factor 7-like-2	*TCF7L2*	rs7903146	Intron	C/T	48	46	7	29.7

MAF: minor allele frequency; MM: major allele homozygous; Mm: heterozygous; mm: minor allele homozygous.

5.3.6 Statistical analyses

Statistical analyses were performed using SAS 9.4 (SAS, Cary, NC) based on a per protocol approach. Normality was assessed using the Shapiro–Wilk test and the skewness value. Non-normally distributed variables were log-transformed before analysis. The results are expressed as least square means ± SEMs unless otherwise specified and statistical significance was set at *P*-value < 0.05. PROC MIXED with repeated-measure procedure was used to assess the effect of the three dietary treatments on IR and glycemic homeostasis. Treatment, sex, age, and genotype were used as fixed effects, participants as a repeated factor. Random effects were identified as treatment sequence, clinical site, and participant. Pre-specified potential confounders such as ethnicity, baseline body composition, baseline fasting glucose level, and HOMA-IR were investigated in all models. The Hardy-Weinberg equilibrium was assessed with χ^2 test. The effects of possible gene-MUFA interactions on IR and glycemic homeostasis were assessed using the same approach. However, due to the considerably comparable MUFA concentrations in the two canola treatments compared to the high-SFA treatment, the statistical analysis of the interaction between diet and genetic polymorphism was conducted to compare the combined effect of the two MUFA diets versus the high-SFA diet. In addition to the analysis of the effect of each SNP separately, possible effects of their interaction on IR and glycemic homeostasis was also evaluated using the same approach.

5.4 Results

A total of 125 participants completed the trial. Three participants were excluded due to high fasting blood glucose levels (>7.0 mmol/L), and six participants were excluded due to large body weight changes (>5% endpoint-baseline weight change), therefore, 116 participants (72

women and 44 men) were included in this study of the effect of FA composition on IR and glycemic homeostasis. Participant characteristics are presented in **Table 5.2**. All participants were non-diabetic and mean HOMA-IR was above 3.0 at baseline (**Table 5.2**) as well as at endpoint of dietary interventions (**Table 5.3**). No differences were observed in fasting concentrations of glucose, insulin, or fructosamine, nor HOMA-IR or HOMA-β indices following the consumption of HOCO or RCO compared to the high-SFA treatment (**Table 5.3**).

Only 101 participants (60 women and 41 men) provided consent for genetic analyses. The ethnicity for the majority (73%) of participants was Caucasian. Genotype-frequencies did not differ from Hardy–Weinberg equilibrium except for the *ADIPOQ* rs266729. The genotype associations with glycemic response to different dietary MUFA levels (**Table 5.4**) showed that consumption of the high-SFA diet induced reductions, compared to the combined high-MUFA diets, in insulin (high-SFA: 11.8 ± 3.6 and high-MUFA: 13.6 ± 3.5, p= 0.04), HOMA-β (high-SFA: 151 ± 46 and high-MUFA: 178 ± 43, p= 0.026), and a tendency in HOMA-IR (high-SFA: 2.6 ± 0.9 and high-MUFA: 3.1 ± 0.9, p= 0.07) in the *IRS1* rs7578326-GG homozygotes.

Table 5.2: Characteristics of participants at baseline of the dietary intervention[1]

Characteristic	Total (n=116)	Female (n=72)	Male (n=44)	*P*[2]
Age (years)	43.9 ± 1.2	45.8 ± 1.5	40.8 ± 1.9	0.0416
Waist circumference (cm)	104.6 ± 1.2	102.2 ± 1.4	108.5 ± 1.8	0.0071
Body weight (kg)	90.2 ± 1.7	83.9 ± 2.0	99.8 ± 2.5	<.0001
BMI[3] (kg/m^2)	31.3 ± 0.5	31.4 ± 0.6	31.3 ± 0.8	0.9197
Systolic BP (mmHg)	119.4 ± 1.2	118.7 ± 1.6	120.5 ± 2.0	0.4679
Diastolic BP (mmHg)	78.6 ± 1.0	78.3 ± 1.3	79.2 ± 1.6	0.673
Cholesterol (mmol/L)	5.18 ± 0.08	5.19 ± 0.11	5.17 ± 0.14	0.9377
Triglycerides (mmol/L)	1.58 ± 0.07	1.51 ± 0.09	1.70 ± 0.11	0.1604
HDL-cholesterol (mmol/L)	1.34 ± 0.03	1.43 ± 0.04	1.19 ± 0.05	0.0004
LDL-cholesterol (mmol/L)	3.12 ± 0.07	3.07 ± 0.09	3.21 ± 0.11	0.3538
Glucose (mg/dl)	94.32 ± 0.72	94.50 ± 1.08	94.14 ± 1.26	0.8913
Insulin (μIU/ml)	13.88 ± 0.78	13.45 ± 0.99	14.61 ± 1.27	0.4723
HOMA IR	3.94 ± 0.25	3.80 ± 0.31	4.16 ± 0.41	0.4791
HOMA-β	198.4 ± 10.1	190.2 ± 12.8	212.1 ± 16.5	0.2979

[1]All values are means ± SEMs unless otherwise specified.
[2]SAS PROC MIXED procedure used to assess the inter-sex differences, P<0.05 was considered significant. SAS PROC MEANS used to determine the mean characteristics of the overall population.
[3]BP: blood pressure; BMI: body mass index; HDL: high-density lipoprotein; LDL: low-density lipoprotein; HOMA-β: homeostasis model assessment of beta-cell function; HOMA-IR: homeostasis model assessment of insulin resistance.

Table 5.3: Fasting insulin, glucose, fructosamine, and homeostatic model assessment of insulin sensitivity and β-cell function according to dietary intervention[1]

Variable	Total (n=116)				Female (n=72)			Male (n=44)			
	HOCO	RCO	Control	P^2	HOCO	RCO	Control	HOCO	RCO	Control	P^a
Insulin (μIU/ml)	16.5 ± 2.4	16.4 ± 2.4	16.8 ± 2.4	0.89	16.1 ± 2.5	15.7 ± 2.5	16.2 ± 2.5	16.9 ± 2.6	17.2 ± 2.6	17.4 ± 2.6	0.28
Glucose (mg/dl)	93.9 ± 1.1	93.3 ± 1.1	94.5 ± 1.1	0.08	93.7 ± 1.3	92.9 ± 1.3	94.0 ± 1.3	94.1 ± 1.6	93.7 ± 1.5	5.3 ± 1.5	0.80
Fructosamine (μmol/l)	224.3 ± 2.3	223.8 ± 2.3	224.7 ± 2.3	0.63	220.9 ± 2.5	219.5 ± 2.5	220.6 ± 2.5	227.8 ± 2.9	228.2 ± 2.9	228.7 ± 2.9	0.63
HOMA-IR	3.89 ± 0.57	3.84 ± 0.57	4.00 ± 0.57	0.72	3.81 ± 0.59	3.70 ± 0.59	3.89 ± 0.59	3.96 ± 0.64	3.98 ± 0.64	4.10 ± 0.64	0.31
HOMA-β	203.7 ± 31.1	208.3 ±31.1	198.9 ± 31.1	0.45	196.4 ± 31.9	193.3 ±31.9	194.6 ± 31.9	211.1 ± 33.5	223.3 ±33.5	203.1 ±33.5	0.43

[1]All data represent the endpoint measures and are presented as least-squares means ± SEMs. HOCO: high-oleic canola; HOMA-β: homeostatic model assessment of β-cell function; HOMA-IR: homeostatic model assessment of insulin resistance; MUFA: monounsaturated fatty acids; RCO: regular canola oil.
[2]SAS PROC MIXED with repeated measure procedure used to assess the effect of MUFA consumption on the reported glycemic control-related measures, using participants as a repeated factor. Treatment, sex, age, and genotype were used as fixed effects. Random effects were treatment sequence, clinical site, and participants. $P<0.05$ was considered significant. P: the difference between the treatments in the overall population. P^a: P of gender-by-treatment interaction.

No effects of different dietary MUFA levels on IR or glycemic homeostasis were observed in the carriers of the *IRS1* rs7578326-A allele. The *IRS1* rs2943641 polymorphism did not modify IR or glycemic responsiveness to MUFA modification.

The analysis of the combination of SNPs rs2943641 and rs7578326 within *IRS1* yielded three genotype combinations; rs2943641-C + rs7578326-A (n=92), rs2943641-TT + rs7578326-A (n=2), and rs2943641-TT + rs7578326-GG (n=7). The *IRS1* rs2943641-TT + rs7578326-A combination was excluded from the final statistical analysis due to low frequency. The combination of rs2943641-TT and rs7578326-GG genotypes reduced insulin high-SFA: 11.8 ± 3.7 and high-MUFA: 13.7 ± 3.6, p= 0.034), HOMA-β (high-SFA: 150.7 ± 46.3 and high-MUFA: 178.3 ± 43.6, p= 0.023), and HOMA-IR (high-SFA: 2.6 ± 1.0 and high-MUFA: 3.1 ± 0.9, p= 0.06) levels in response to the high-SFA diet compared to higher MUFA consumption (**Figure 5.1**). Measures of IR and glycemic control did not respond to the dietary intervention in the carriers of the combination of the *IRS1* rs2943641-C + rs7578326-A.

The results also revealed a reduction (high-SFA: 4.3 ± 0.9 and high-MUFA: 4.5 ± 0.8, p= 0.042) in HOMA-IR and a concomitant reduction (high-SFA: 16.7 ± 3.3 and high-MUFA: 17.9 ± 3.2, p= 0.06) in fasting insulin concentrations following consumption of the high-SFA diet compared to the high-MUFA diets in the *FABP1* rs2241883-CC homozygotes. No significant FA-by-gene interactions were observed in the other six candidate SNPs within *ADIPOQ*, *ADRB2*, *FTO*, *PLIN1*, *PPARγ*, and *TCF7L2* on the responses of fasting insulin, fasting glucose, HOMA-IR, and HOMA-β to dietary FA modification. Furthermore, none of the nine selected polymorphisms modulated fructosamine levels in response to dietary MUFA (data not shown).

Table 5.4: Fasting insulin, glucose, and homeostatic model assessment of insulin sensitivity and β-cell function according to dietary intervention and selected polymorphisms[1]

Gene, SNP	Allele (n)	Insulin (μIU/ml)			Glucose (mg/dl)			HOMA-IR			HOMA-β		
		High MUFA	Low MUFA	*P*[2]	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*
ADIPOQ rs266729	CC (61)	16.5 ± 2.3	17.0 ± 2.3		93.4 ± 1.3	94.7 ± 1.3		3.8 ± 0.6	4.1 ± 0.6		208 ± 29	199 ± 29	
	CG (30)	15.9 ± 2.5	15.1 ± 2.6	0.42	91.7 ± 1.6	90.9 ± 1.7	0.08	3.6 ± 0.6	3.4 ± 0.6	0.29	208 ± 31	211 ± 32	0.74
	GG (10)	17.6 ± 3.2	17.8 ± 3.3		95.4 ± 2.6	97.3 ± 2.8		4.3 ± 0.8	4.4 ± 0.8		194 ± 39	184 ± 41	
ADRB2 rs1042714	CC (42)	16.0 ± 2.4	16.3 ± 2.4		94.7 ± 1.4	95.3 ± 1.5		3.8 ± 0.6	3.9 ± 0.6		184 ± 28	178 ± 29	
	CG (41)	17.4 ± 2.4	17.3 ± 2.4	0.84	93.5 ± 1.4	94.0 ± 1.5	0.82	4.1 ± 0.6	4.1 ± 0.6	0.79	215 ± 28	209 ± 29	1.00
	GG (18)	15.2 ± 2.8	15.6 ± 2.8		88.6 ± 2.0	90.1 ± 2.1		3.4 ± 0.7	3.5 ± 0.7		235 ± 33	233 ± 34	
FABP1 rs2241883	CC (10)	17.9 ± 3.2	16.7 ± 3.3‡		97.6 ± 2.7	95.3 ± 2.8		4.5 ± 0.8	4.3 ± 0.9*		180 ± 39	171 ± 41	
	TC (45)	15.5 ± 2.4	16.0 ± 2.4	0.14	92.2 ± 1.4	93.4 ± 1.4	0.06	3.6 ± 0.6	3.7 ± 0.6	0.08	203 ± 29	197 ± 30	0.95
	TT (46)	17.0 ± 2.4	17.2 ± 2.4		92.9 ± 1.4	93.9 ± 1.4		3.9 ± 0.6	4.0 ± 0.6		216 ± 29	212 ± 30	
FTO rs9939609	AA (18)	18.6 ± 2.9	17.7 ± 2.9		91.2 ± 2.1	92.3 ± 2.2		4.2 ± 0.7	4.0 ± 0.7		268 ± 36	232 ± 37	
	AT (39)	15.5 ± 2.5	15.3 ± 2.5	0.76	94.5 ± 1.5	95.3 ± 1.6	0.84	3.7 ± 0.6	3.7 ± 0.6	0.84	177 ± 32	170 ± 33	0.38
	TT (44)	16.5 ± 2.5	17.4 ± 2.5		92.7 ± 1.5	93.2 ± 1.5		3.8 ± 0.6	4.1 ± 0.6		210 ± 31	218 ± 32	
IRS1 rs2943641	CC (50)	16.2 ± 2.3	15.7 ± 2.4		92.5 ± 1.4	92.8 ± 1.4		3.8 ± 0.6	3.6 ± 0.6		204 ± 28	192 ± 29	
	CT (42)	17.1 ± 2.4	18.3 ± 2.4	0.13	93.9 ± 1.4	95.2 ± 1.5	0.72	4.0 ± 0.6	4.4 ± 0.6	0.13	213 ± 29	218 ± 29	0.27
	TT (9)	13.8 ± 3.3	13.1 ± 3.4		92.7 ± 2.8	93.0 ± 3.0		3.1 ± 0.9	2.9 ± 0.9		173 ± 40	161 ± 43	
IRS1 rs7578326	AA (49)	15.4 ± 2.3	14.6 ± 2.3		92.8 ± 1.4	93.1 ± 1.4		3.6 ± 0.6	3.4 ± 0.6		194 ± 28	181 ± 29	
	AG (45)	17.7 ± 2.3	19.2 ± 2.3	0.01	93.7 ± 1.4	94.8 ± 1.5	0.87	4.2 ± 0.6	4.6 ± 0.6	0.02	221 ± 28	228 ± 29	0.03
	GG (7)	13.6 ± 3.5	11.8 ± 3.6*		91.9 ± 3.2	92.5 ± 3.3		3.1 ± 0.9	2.6 ± 0.9‡		178 ± 43	151 ± 46*	

Table 5.4: Continued

Gene, SNP	Allele (n)	Insulin (μIU/ml)			Glucose (mg/dl)			HOMA-IR			HOMA-β		
		High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*	High MUFA	Low MUFA	*P*
PLIN rs894160	CC (47)	16.4 ± 2.4	16.5 ± 2.4		94.2 ± 1.4	94.8 ± 1.5		3.8 ± 0.6	3.9 ± 0.6		196 ± 28	191 ± 29	
	CT (43)	16.9 ± 2.4	16.8 ± 2.4	0.57	92.0 ± 1.4	92.7 ± 1.5	0.78	3.9 ± 0.6	4.0 ± 0.6	0.55	222 ± 28	214 ± 29	0.88
	TT (11)	14.9 ± 3.1	16.1 ± 3.2		93.5 ± 2.5	95.0 ± 2.7		3.4 ± 0.8	3.8 ± 0.8		183 ± 37	185 ± 39	
PPARγ rs1801282	CC (78)	16.0 ± 2.3	16.3 ± 2.3		92.2 ± 1.0	92.7 ± 1.1		3.7 ± 0.6	3.8 ± 0.6		208 ± 28	205 ± 28	
	CG (21)	17.4 ± 2.7	17.1 ± 2.8	0.54	95.5 ± 1.8	96.7 ± 1.9	0.72	4.1 ± 0.7	4.1 ± 0.7	0.66	201 ± 33	187 ± 34	0.36
	GG (2)	21.3 ± 5.9	21.1 ± 6.1		105.0±5.6	107.7 ± 5.9		5.6 ± 1.6	5.6 ± 1.6		193 ± 71	176 ± 77	
TCF7L2 rs7903146	CC (48)	15.8 ± 2.4	15.7 ± 2.4		92.1 ± 1.3	92.2 ± 1.3		3.6 ± 0.6	3.6 ± 0.6		198 ± 29	200 ± 30	
	CT (46)	17.0 ± 2.4	17.7 ± 2.4	0.95	94.2 ± 1.3	95.5 ± 1.4	0.31	4.0 ± 0.6	4.3 ± 0.6	0.97	215 ± 30	204 ± 30	0.38
	TT (7)	17.8 ± 3.6	16.0 ± 3.7		93.9 ± 3.1	95.5 ± 3.2		4.2 ± 0.9	3.7 ± 1.0		209 ± 44	189 ± 47	

[1]All data represent the endpoint measures and are presented as least-squares means ± SEMs. n=101 participants. *ADIPOQ*: adiponectin; *ADRB2*: adrenoceptor beta-2; *FABP1*: liver fatty acid binding protein; *FTO*: fat mass and obesity-associated gene; HOMA-β: homeostatic model assessment of b-cell function; HOMA-IR: homeostatic model assessment of insulin resistance; *IRS1*: insulin receptor substrate-1; MUFA: monounsaturated fatty acids; *PLIN*: perilipin-1; *PPARγ*: peroxisome proliferator-activated receptor gamma; SNP: single nucleotide polymorphism; *TCF7L2*: transcription factor 7-like 2.
[2]SAS PROC MIXED with repeated measure procedure was used to assess the effect of gene-MUFA interactions on glycemic homeostasis and HOMA indices, using participants as a repeated factor. Treatment, sex, age, and genotype were used as fixed effects. Random effects were treatment sequence, clinical site, and participants. $P < 0.05$ was considered significant. *P*-values presented in the table refer to overall gene-diet interaction. * Indicates significant difference between the consumption of high-SFA versus high-MUFA within the same genotype. ‡Indicates trend toward significance ($p<0.07$) between the consumption of high-SFA versus high-MUFA within the same genotype.

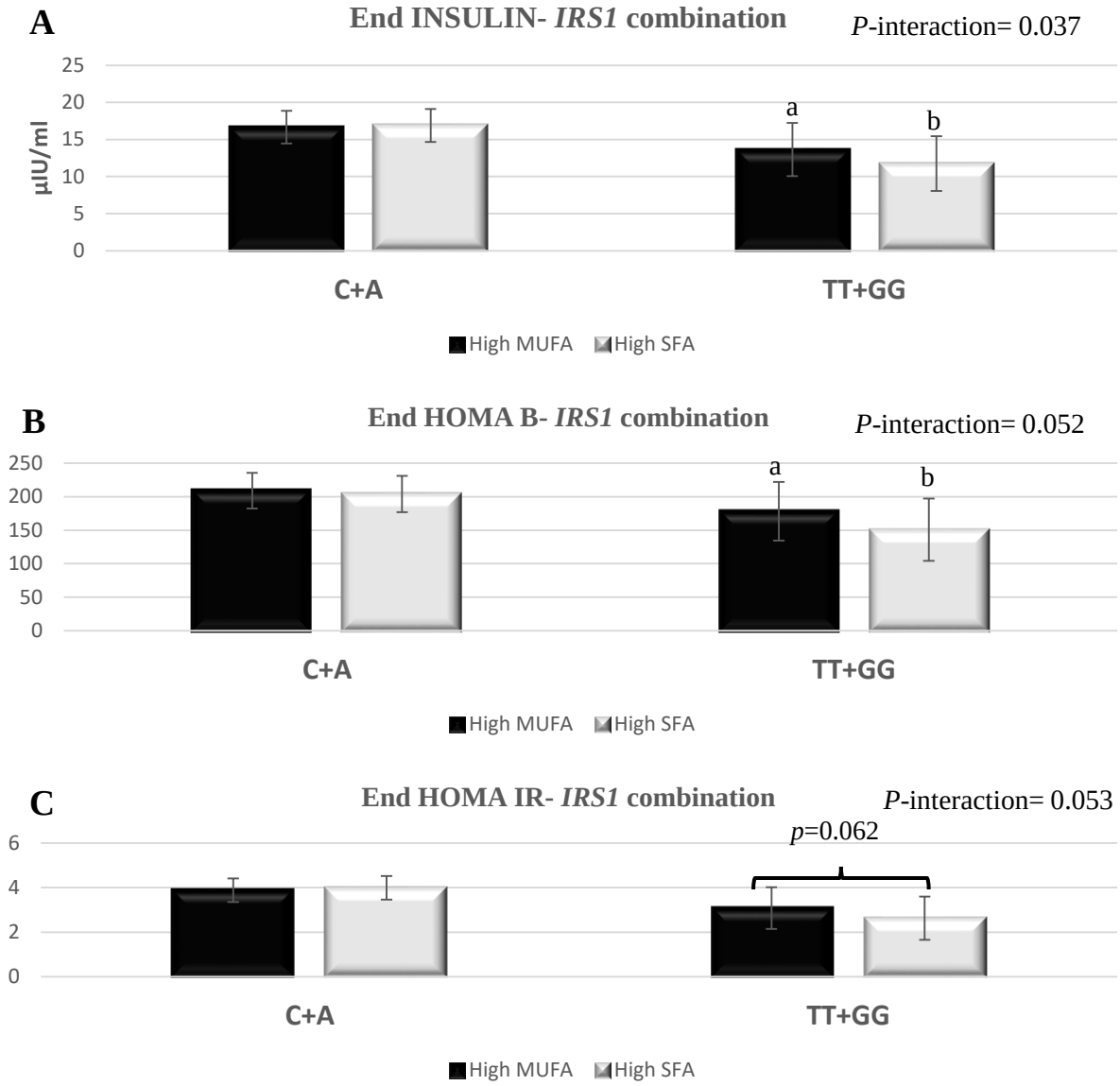

Figure 5.1: End-point responses of insulin (A), HOMA-β (B), and HOMA-IR (C) to dietary MUFA by *IRS1* rs2943641 + rs7578326 combination. SAS PROC MIXED with repeated measure procedure used to assess the effect of gene-MUFA interactions on IR and glycemic response, using participants as a repeated factor. Treatment, sex, age, and genotype were used as fixed effects. Random effects were treatment sequence, clinical site, and participants. P<0.05 was considered significant. Values are least-squares means ± SEMs. Labeled bars with different lowercase letters significantly differ, P < 0.05. HOMA: homeostasis model assessment of insulin resistance (IR) and cell function (β); *IRS1*: insulin receptor substrate-1 gene; *IRS1* combination: rs2943641-C/T and rs7578326-A/G; MUFA: monounsaturated fatty acid; SFA: saturated fatty acid. Frequency of SNP combination (n=99): C+A= 93% and TT+GG= 7%.

5.5 Discussion

Genetic architecture may modulate insulin sensitivity responses to dietary FA composition. This study revealed beneficial effects of a low-MUFA high-SFA fat consumption pattern on IR measures in the *IRS1* rs7578326-GG homozygotes and, for the first time, to our knowledge, in the carriers of the *FABP1* rs2241883-CC genotypes compared to the high-MUFA diets. These results may, partly, explain the inter-individual variability in the responsiveness of IR to dietary FA modifications.

The current report failed to find overall favorable effects of high-MUFA consumption on IR measures in these non-diabetic subjects with abdominal obesity. Consumption of MUFA-rich diets (>20% of total energy) for three months (3) and six months (7) has been previously reported to ameliorate HOMA-IR compared to SFA-rich diet (>15% of total energy). However, studies with shorter durations showed contradictory results regarding the effects of MUFA on glucose homeostasis and IR (11, 12). Therefore, the six-week duration of the current study might have been insufficient to permit identification of the proposed beneficial effect of MUFA on IR and glucose homeostasis in our participants with abdominal obesity. Further, genetic predisposition and, possibly, interactions with dietary FAs may modify the individual response to dietary intervention. For instance, even though SFA consumption has been repeatedly reported to be positively associated with IR and hyperinsulinemia (2, 3, 7), Luan et al. showed a genetically-oriented response of fasting insulin concentrations to dietary SFA intake mediated by the *PPARγ* rs1801282 polymorphism (27). Similarly, the present study results shed light on genetically-driven responses of IR measures to dietary FA composition.

The rs2943641 and rs7578326 polymorphisms within *IRS1* have been reported to modulate IR in different ethnic populations (1, 17, 18, 28). The *IRS1* rs2943641 is the best-

associated variant with type 2 diabetes and IR in *IRS1* locus (1, 29), and has been marked as a potential functional variant (30). While the function of the *IRS1* rs7578326 is not defined yet, the A-allele was found to be associated with a higher risk for type 2 diabetes (20, 31). The latter polymorphism is located in the intron region which might coordinate splicing and hence the expression of the IRS1 protein. The upregulation of *IRS1* expression might alleviate IR and restore the impairment of insulin signaling pathways (21).

Both *IRS1* variants have been reported to modulate the effects of quantity and quality of dietary macronutrients on IR and type 2 diabetes (1, 20, 32). Zheng et al. reported that the rs7578326 and rs2943641 polymorphisms within the *IRS1* gene modified IR in response to the SFA-to-carbohydrate ratio and MUFA consumption in two independent populations (20). In the present study, we found an improvement in insulin sensitivity, measured by reductions in fasting insulin, HOMA-IR, and HOMA-β, in the *IRS1* rs7578326-GG genotype carriers following consumption of the high-SFA low-MUFA diet compared to the high-MUFA diets in a controlled feeding trial. This finding supports the aforementioned study by Zheng et al. which reported that the carriers of the rs7578326-G allele who consumed a low-MUFA diet incurred a significant reduction in HOMA-IR compared to the non-carriers (20); however, the reported low-MUFA consumption in their study might reflect high-SFA and/or high-PUFA. A potential mechanism of this interaction might be related to lipid-induced modulatory effect of tyrosine phosphorylation (33); however, further research is required to reveal the mechanism(s) that coordinate the interaction between dietary FA composition and *IRS1* polymorphisms on IR.

The results of this study also indicate that the homozygotes for the combination of rs2943641-TT and rs7578326-GG of *IRS1* (frequency= 7%) may have improved insulin sensitivity following high-SFA compared to high-MUFA consumption. The *IRS1* rs7578326 is

adjacent and in linkage disequilibrium with the potential functional *IRS1* rs2943641 (r^2= 0.79, in HapMap CEU) (29). Therefore, the modulatory effect of the combined the rs2943641-TT and rs7578326-GG *IRS1* genotypes on IR might suggest that the rs7578326 regulate insulin signaling through the functional rs2943641. This finding supports the previously reported modulatory effect of the combination of *IRS1* rs7578326-G and rs2943641-T alleles on IR response to dietary intervention (20).

The functional *FABP1* rs2241883 (T94A) was found to be associated with type 2 diabetes and IR (16, 34). The physiological role of FABP1, as a key regulator of lipid metabolism, is suggested to be optimized by the presence of threonine at the N-terminal region of the FA binding site (position 94) (34, 35). The rs2241883 mutation (A94/ C allele) could reduce the binding capacity of FABP1 to long-chain FA and subsequently, alter normal lipid metabolism (34). However, the responsiveness of the *FABP1* A94 allele to dietary FA composition has been previously reported (36). In the current study, consumption of the high-SFA diet in the *FABP1* rs2241883-CC homozygotes ameliorated IR compared to non-carriers. Actually, MCFA contributed to ~18% (10.9 g) of the high-SFA treatment of this study compared to negligible amount in the high-MUFA treatments. The consumption of small amount of MCFAs (≤ 10-18 g/d) for ≤ 90 days has been previously reported to induce beneficial physiological effects including reductions in body weight and fat mass (37, 38), improvement in lipid profile (38), as well as amelioration of HOMA-IR (39). Accordingly, in this study, MCFAs could partly counteract the *FABP1* rs2241883-CC genotype (A94A)-associated proposed reduction in the activity of FABP1 thus, inducing beneficial effects on IR. MCFAs, unlike long-chain FAs, are freely permeable into cells and mitochondria, therefore, a reduction in *FABP1* activity may not inhibit their cellular uptake and/or metabolism as well as would reduce FA flux

to the liver (40, 41). Consequently, we suggest that MCFAs, despite being present in small concentrations, in the high-SFA diet might improve IR by reducing FA flux to the liver in the *FABP1* rs2241883-CC genotype carriers, thereby, probably overriding the effects of other SFA on glucose insulin homeostasis. To the best of our knowledge, this is the first study to evaluate the effect of FA composition-by-*FABP1* rs2241883 interaction on IR, therefore, confirmatory and biochemical studies are required.

In this study, polymorphisms within *ADIPOQ*, *ADRB2*, *FTO*, *PLIN1*, *PPARγ*, and *TCF7L2* genes failed to modulate the effect of MUFA consumption on glucose homeostasis and IR measures. However, conflicting data still exist regarding IR responsiveness to diet-by-gene interactions (25, 42-44). A major strength of the present study is the crossover design with a controlled full-feeding diet and a relatively large number of participants for such a dietary intervention. In the present study, insulin resistance was assessed by HOMA-IR rather than using the gold standard euglycemic glucose clamp technique which might be considered as a limitation. However, HOMA-IR is a feasible and robust tool for the surrogate assessment of IR, especially in non-diabetic subjects, and has been validated across the clamp technique (45). Although the majority (73%) of study participants was Caucasian, the mixed ethnicity might be considered as another limitation; however, it might, in contrast, provide generalizability of the current findings to the population at large.

In conclusion, polymorphisms in *FABP1* and *IRS1* appeared to modulate the IR response to dietary FA composition. The observed interactions between dietary FA composition and genetic variants within *FABP1* and *IRS1* genes on HOMA-IR may reflect a potential clinical benefit on insulin sensitivity following prolonged exposure to dietary FA modifications. These

results may eventually contribute to developing an effective genotype-based dietary recommendation to reduce the incidence and associated complications of IR and type 2 diabetes.

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5.6 References

1. Qi Q, Bray GA, Smith SR, Hu FB, Sacks FM, Qi L. Insulin receptor substrate 1 gene variation modifies insulin resistance response to weight-loss diets in a 2-year randomized trial: the Preventing Overweight Using Novel Dietary Strategies (POUNDS LOST) trial. Circulation 2011;124(5):563-71. doi: 10.1161/circulationaha.111.025767.

2. Riccardi G, Giacco R, Rivellese AA. Dietary fat, insulin sensitivity and the metabolic syndrome. Clin Nutr 2004;23(4):447-56. doi: 10.1016/j.clnu.2004.02.006.

3. Vessby B, Uusitupa M, Hermansen K, Riccardi G, Rivellese AA, Tapsell LC, Nalsen C, Berglund L, Louheranta A, Rasmussen BM, et al. Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. Diabetologia 2001;44(3):312-9.

4. Galgani JE, Uauy RD, Aguirre CA, Diaz EO. Effect of the dietary fat quality on insulin sensitivity. Br J Nutr 2008;100(3):471-9. doi: 10.1017/s0007114508894408.

5. Lovejoy JC. The influence of dietary fat on insulin resistance. Curr Diab Rep 2002;2(5):435-40.

6. Turner N, Hariharan K, TidAng J, Frangioudakis G, Beale SM, Wright LE, Zeng XY, Leslie SJ, Li JY, Kraegen EW, et al. Enhancement of muscle mitochondrial oxidative capacity and alterations in insulin action are lipid species dependent: potent tissue-specific effects of medium-chain fatty acids. Diabetes 2009;58(11):2547-54. doi: 10.2337/db09-0784.

7. Due A, Larsen TM, Hermansen K, Stender S, Holst JJ, Toubro S, Martinussen T, Astrup A. Comparison of the effects on insulin resistance and glucose tolerance of 6-mo high-monounsaturated-fat, low-fat, and control diets. Am J Clin Nutr 2008;87(4):855-62.

8. Errazuriz I, Dube S, Slama M, Visentin R, Nayar S, O'Connor H, Cobelli C, Das SK, Basu A, Kremers WK, et al. Randomized controlled trial of a MUFA or fiber-rich diet on hepatic fat in prediabetes. J Clin Endocrinol Metab 2017;102(5):1765-74. doi: 10.1210/jc.2016-3722.

9. Paniagua JA, Gallego de la Sacristana A, Romero I, Vidal-Puig A, Latre JM, Sanchez E, Perez-Martinez P, Lopez-Miranda J, Perez-Jimenez F. Monounsaturated fat-rich diet prevents central body fat distribution and decreases postprandial adiponectin expression induced by a carbohydrate-rich diet in insulin-resistant subjects. Diabetes Care 2007;30(7):1717-23. doi: 10.2337/dc06-2220.

10. Haghighatdoost F, Hosseinzadeh-Attar MJ, Kabiri A, Eshraghian M, Esmaillzadeh A. Effect of substituting saturated with monounsaturated fatty acids on serum visfatin levels and insulin resistance in overweight women: a randomized cross-over clinical trial. Int J Food Sci Nutr 2012;63(7):772-81. doi: 10.3109/09637486.2012.665044.

11. Xiao C, Giacca A, Carpentier A, Lewis GF. Differential effects of monounsaturated, polyunsaturated and saturated fat ingestion on glucose-stimulated insulin secretion, sensitivity and clearance in overweight and obese, non-diabetic humans. Diabetologia 2006;49(6):1371-9. doi: 10.1007/s00125-006-0211-x.

12. Lovejoy JC, Smith SR, Champagne CM, Most MM, Lefevre M, DeLany JP, Denkins YM, Rood JC, Veldhuis J, Bray GA. Effects of diets enriched in saturated (palmitic), monounsaturated (oleic), or trans (elaidic) fatty acids on insulin sensitivity and substrate oxidation in healthy adults. Diabetes Care 2002;25(8):1283-8.

13. Chang LF, Vethakkan SR, Nesaretnam K, Sanders TA, Teng KT. Adverse effects on insulin secretion of replacing saturated fat with refined carbohydrate but not with monounsaturated fat: A randomized controlled trial in centrally obese subjects. J Clin Lipidol 2016;10(6):1431-41.e1. doi: 10.1016/j.jacl.2016.09.006.

14. Fall T, Ingelsson E. Genome-wide association studies of obesity and metabolic syndrome. Mol Cell Endocrinol 2014;382(1):740-57. doi: 10.1016/j.mce.2012.08.018.

15. Brown AE, Walker M. Genetics of insulin resistance and the metabolic syndrome. Curr Cardiol Rep 2016;18(8):75. doi: 10.1007/s11886-016-0755-4.

16. Mansego ML, Martinez F, Martinez-Larrad MT, Zabena C, Rojo G, Morcillo S, Soriguer F, Martin-Escudero JC, Serrano-Rios M, Redon J, et al. Common variants of the liver fatty acid binding protein gene influence the risk of type 2 diabetes and insulin resistance in Spanish population. PLoS One 2012;7(3):e31853. doi: 10.1371/journal.pone.0031853.

17. Li Q, Qiao Y, Wang C, Zhang G, Zhang X, Xu L. Associations between two single-nucleotide polymorphisms (rs1801278 and rs2943641) of insulin receptor substrate 1 gene and type 2 diabetes susceptibility: a meta-analysis. Endocrine 2016;51(1):52-62. doi: 10.1007/s12020-015-0770-z.

18. Alharbi KK, Khan IA, Munshi A, Alharbi FK, Al-Sheikh Y, Alnbaheen MS. Association of the genetic variants of insulin receptor substrate 1 (IRS-1) with type 2 diabetes mellitus in a Saudi population. Endocrine 2014;47(2):472-7. doi: 10.1007/s12020-014-0177-2.

19. Sesti G, Federici M, Hribal ML, Lauro D, Sbraccia P, Lauro R. Defects of the insulin receptor substrate (IRS) system in human metabolic disorders. Faseb j 2001;15(12):2099-111. doi: 10.1096/fj.01-0009rev.

20. Zheng JS, Arnett DK, Parnell LD, Smith CE, Li D, Borecki IB, Tucker KL, Ordovas JM, Lai CQ. Modulation by dietary fat and carbohydrate of IRS1 association with type 2 diabetes traits in two populations of different ancestries. Diabetes Care 2013;36(9):2621-7. doi: 10.2337/dc12-2607.

21. Cheng L, Song J, Li G, Liu Y, Wang Y, Meng X, Sun G, Sun X. Effects of the Tangningtongluo formula as an alternative strategy for diabetics via upregulation of insulin receptor substrate-1. Mol Med Rep 2017;16(1):703-9. doi: 10.3892/mmr.2017.6679.

22. McIntosh AL, Huang H, Storey SM, Landrock KK, Landrock D, Petrescu AD, Gupta S, Atshaves BP, Kier AB, Schroeder F. Human FABP1 T94A variant impacts fatty acid metabolism and PPAR-alpha activation in cultured human female hepatocytes. Am J Physiol Gastrointest Liver Physiol 2014;307(2):G164-76. doi: 10.1152/ajpgi.00369.2013.

23. Newberry EP, Xie Y, Kennedy SM, Luo J, Davidson NO. Protection against Western diet-induced obesity and hepatic steatosis in liver fatty acid-binding protein knockout mice. Hepatology 2006;44(5):1191-205. doi: 10.1002/hep.21369.

24. Corella D, Qi L, Tai ES, Deurenberg-Yap M, Tan CE, Chew SK, Ordovas JM. Perilipin gene variation determines higher susceptibility to insulin resistance in Asian women when consuming a high-saturated fat, low-carbohydrate diet. Diabetes Care 2006;29(6):1313-9. doi: 10.2337/dc06-0045.

25. Zheng Y, Huang T, Zhang X, Rood J, Bray GA, Sacks FM, Qi L. Dietary fat modifies the effects of FTO genotype on changes in insulin sensitivity. J Nutr 2015;145(5):977-82. doi: 10.3945/jn.115.210005.

26. Song Y, Manson JAE, Tinker L, Howard BV, Kuller LH, Nathan L, Rifai N, Liu S. Insulin sensitivity and insulin secretion determined by homeostasis model assessment (HOMA) and risk of diabetes in a multiethnic cohort of women: The Women's Health Initiative Observational Study. Diabetes Care 2007;30(7):1747-52. doi: 10.2337/dc07-0358.

27. Luan J, Browne PO, Harding AH, Halsall DJ, O'Rahilly S, Chatterjee VK, Wareham NJ. Evidence for gene-nutrient interaction at the PPARgamma locus. Diabetes 2001;50(3):686-9.

28. Rosta K, Al-Aissa Z, Hadarits O, Harreiter J, Nadasdi A, Kelemen F, Bancher-Todesca D, Komlosi Z, Nemeth L, Rigo J, Jr., et al. Association study with 77 SNPs confirms the robust role for the rs10830963/G of MTNR1B variant and identifies two novel associations in gestational diabetes mellitus development. PLoS One 2017;12(1):e0169781. doi: 10.1371/journal.pone.0169781.

29. Yiannakouris N, Cooper JA, Shah S, Drenos F, Ireland HA, Stephens JW, Li KW, Elkeles R, Godsland IF, Kivimaki M, et al. IRS1 gene variants, dysglycaemic metabolic changes and type-2 diabetes risk. Nutr Metab Cardiovasc Dis 2012;22(12):1024-30. doi: 10.1016/j.numecd.2011.05.009.

30. Rung J, Cauchi S, Albrechtsen A, Shen L, Rocheleau G, Cavalcanti-Proenca C, Bacot F, Balkau B, Belisle A, Borch-Johnsen K, et al. Genetic variant near IRS1 is associated with type 2 diabetes, insulin resistance and hyperinsulinemia. Nat Genet 2009;41(10):1110-5. doi: 10.1038/ng.443.

31. Yoshiuchi I. Evidence of selection at insulin receptor substrate-1 gene loci. Acta Diabetol 2013;50(5):775-9. doi: 10.1007/s00592-012-0414-1.

32. Ericson U, Rukh G, Stojkovic I, Sonestedt E, Gullberg B, Wirfalt E, Wallstrom P, Orho-Melander M. Sex-specific interactions between the IRS1 polymorphism and intakes of carbohydrates and fat on incident type 2 diabetes. Am J Clin Nutr 2013;97(1):208-16. doi: 10.3945/ajcn.112.046474.

33. Samuel VT, Petersen KF, Shulman GI. Lipid-induced insulin resistance: unravelling the mechanism. Lancet 2010;375(9733):2267-77. doi: 10.1016/s0140-6736(10)60408-4.

34. Xue H, Zhao H, Liu X, Zhao YR, Chen ZJ, Ma J. Association of single-nucleotide polymorphisms rs2197076 and rs2241883 of FABP1 gene with polycystic ovary syndrome. J Assist Reprod Genet 2016;33(1):75-83. doi: 10.1007/s10815-015-0626-8.

35. Brouillette C, Bosse Y, Perusse L, Gaudet D, Vohl MC. Effect of liver fatty acid binding protein (FABP) T94A missense mutation on plasma lipoprotein responsiveness to treatment with fenofibrate. J Hum Genet 2004;49(8):424-32. doi: 10.1007/s10038-004-0171-2.

36. Robitaille J, Brouillette C, Lemieux S, Perusse L, Gaudet D, Vohl MC. Plasma concentrations of apolipoprotein B are modulated by a gene--diet interaction effect between the LFABP T94A polymorphism and dietary fat intake in French-Canadian men. Mol Genet Metab 2004;82(4):296-303. doi: 10.1016/j.ymgme.2004.06.002.

37. Tsuji H, Kasai M, Takeuchi H, Nakamura M, Okazaki M, Kondo K. Dietary medium-chain triacylglycerols suppress accumulation of body fat in a double-blind, controlled trial in healthy men and women. J Nutr 2001;131(11):2853-9.

38. Kasai M, Nosaka N, Maki H, Negishi S, Aoyama T, Nakamura M, Suzuki Y, Tsuji H, Uto H, Okazaki M, et al. Effect of dietary medium- and long-chain triacylglycerols (MLCT) on accumulation of body fat in healthy humans. Asia Pac J Clin Nutr 2003;12(2):151-60.

39. Han JR, Deng B, Sun J, Chen CG, Corkey BE, Kirkland JL, Ma J, Guo W. Effects of dietary medium-chain triglyceride on weight loss and insulin sensitivity in a group of moderately overweight free-living type 2 diabetic Chinese subjects. Metabolism 2007;56(7):985-91. doi: 10.1016/j.metabol.2007.03.005.

40. Atshaves BP, Martin GG, Hostetler HA, McIntosh AL, Kier AB, Schroeder F. Liver fatty acid-binding protein and obesity. J Nutr Biochem 2010;21(11):1015-32. doi: 10.1016/j.jnutbio.2010.01.005.

41. Wein S, Wolffram S, Schrezenmeir J, Gasperikova D, Klimes I, Sebokova E. Medium-chain fatty acids ameliorate insulin resistance caused by high-fat diets in rats. Diabetes Metab Res Rev 2009;25(2):185-94. doi: 10.1002/dmrr.925.

42. Grau K, Hansen T, Holst C, Astrup A, Saris WH, Arner P, Rossner S, Macdonald I, Polak J, Oppert JM, et al. Macronutrient-specific effect of FTO rs9939609 in response to a 10-week randomized hypo-energetic diet among obese Europeans. Int J Obes (Lond) 2009;33(11):1227-34. doi: 10.1038/ijo.2009.159.

43. Warodomwichit D, Shen J, Arnett DK, Tsai MY, Kabagambe EK, Peacock JM, Hixson JE, Straka RJ, Province MA, An P, et al. ADIPOQ polymorphisms, monounsaturated fatty acids, and obesity risk: the GOLDN study. Obesity (Silver Spring) 2009;17(3):510-7. doi: 10.1038/oby.2008.583.

44. Perez-Martinez P, Lopez-Miranda J, Cruz-Teno C, Delgado-Lista J, Jimenez-Gomez Y, Fernandez JM, Gomez MJ, Marin C, Perez-Jimenez F, Ordovas JM. Adiponectin gene variants are associated with insulin sensitivity in response to dietary fat consumption in Caucasian men. J Nutr 2008;138(9):1609-14.

45. Sarafidis PA, Lasaridis AN, Nilsson PM, Pikilidou MI, Stafilas PC, Kanaki A, Kazakos K, Yovos J, Bakris GL. Validity and reproducibility of HOMA-IR, 1/HOMA-IR, QUICKI and McAuley's indices in patients with hypertension and type II diabetes. J Hum Hypertens 2007;21(9):709-16. doi: 10.1038/sj.jhh.1002201.

BRIDGE TO CHAPTER VI

In the context of the evidence discussed in this thesis regarding the effect of MUFA on modulating the fat accumulation, one former study has proposed a beneficial effect of substituting SFA with MUFA on elevating physical activity level. The following chapter shows the results of investigating the effect of high-MUFA consumption in modulating spontaneous physical activity level. Shatha Hammad participated in study coordination and data collection at RCFFN, handled data analyses and interpretation, conducted the statistical analyses, and wrote the following report.

CHAPTER VI

Monounsaturated fatty acids rich diet failed to modulate spontaneous physical activity behavior in subjects with abdominal obesity

6.1 Introduction

Although the etiology of obesity is multifactorial, from an energy balance point of view, energy expenditure should be increased to minimize fat deposition. Physical activity can substantially enhance energy expenditure level, thus reduces obesity and morbidity risk as well as enhances body fat distribution (1, 2). Inactivity can double the obesity risk in adults compared to their peers who participate in a regular moderate-intensity physical activity (3). Unfortunately, the global epidemic of obesity is associated with escalating degrees of sedentary lifestyle (4), therefore, successful weight-control interventions must consider all available options that boost physical activity level.

Dietary fatty acid composition can modulate energy balance; consumption of monounsaturated fatty acids (MUFA) have been frequently reported to contribute a negative energy balance by elevating diet-induced thermogenesis, fat oxidation, and daily energy expenditure, particularly compared to saturated fatty acids (SFA) (5, 6). The question remains whether increasing dietary MUFA would further influence energy balance by increasing non-resting energy expenditure levels. Kien et al. found that substituting palmitic acid with oleic acid augmented physical activity level and increased resting energy expenditure in young healthy

subjects (7). Herein, the objective was to evaluate the effects of high-MUFA diets on spontaneous physical activity behavior compared to a low-MUFA high-SFA diet in a controlled condition in adults with abdominal obesity.

6.2 Methods

6.2.1 Study design and population

This study was part of the Canola Oil Multi-center Intervention Trial II (COMIT II); a randomized, controlled, double-blinded, crossover study aimed to evaluate the effects of MUFA consumption on body composition. The trial was registered at clinicaltrials.gov as NCT02029833. The protocol was reviewed and approved by institutional ethics boards across the participating clinical sites.

Participants aged 20-65 years were included in the trial if they had abdominal obesity as identified with waist circumference of greater than 94 cm for men and 80 cm for women, in addition to at least one of the following metabolic syndrome parameters as secondary inclusion criteria: fasting blood glucose of ≥ 5.6 mmol/L (according to the American Diabetes Association definition for pre-diabetes), TG ≥1.7 mmol/L, HDL-C <1 mmol/L (men) or <1.3 mmol/L (women), and blood pressure ≥130 mmHg (systolic) and/or ≥85 mmHg (diastolic). Individuals were excluded if they had unstable thyroid, kidney, diabetes, or liver disease. Current smokers, pregnant and lactating women, individuals consuming more than two alcoholic beverages per day were not eligible to participate. Written informed consent was obtained from all participants prior to enrollment.

6.2.2 Dietary intervention

This study consisted of three, six-week feeding periods separated by six-week washout periods, the washout peroids ranged from four to 12-week in some cases to meet the participants needs. During the feeding periods, participants were provided with an iso-caloric, weight maintenance diet containing 35% fat, 50% carbohydrate, and 15% protein of total energy. All three phases were identical except for the type of treatment oil provided. All meals were prepared based on a seven-d rotating menu cycle in the metabolic kitchen of the participating sites. During the washout periods, participants were instructed to consume their habitual diets.

Treatment oils consisted of 20% of total energy and were incorporated into two equal portions of smoothie beverages consumed at breakfast and supper. Treatment oils included: 1) regular canola oil (RCO) consisting of 6.6% SFA, 65.3% MUFA, 19.6% n-6 PUFA, 8.5% α-linolenic acid, 2) high-oleic acid canola oil (HOCO) consisting of 6.7% SFA, 75.9% MUFA, 14.8% n-6 PUFA, 2.6% α-linolenic acid, and 3) a low-MUFA high-SFA control oil consisting of 22.1% long-chain SFA, 18.1% medium-chain FA (MCFA), 22.0% MUFA, 29.6% n-6 PUFA, 8.2% n-3 PUFA α-linolenic acid. The low-MUFA oil blend was prepared using 34.9% safflower oil, 36.0% ghee/butter oil, 16.0% coconut oil, and 13.1% flaxseed oil. Compliance was assessed by the consumption of smoothies; 90% success was the required target for smoothie consumption at each phase. Participants signed a daily checklist to verify smoothies' consumption. To ensure optimal compliance, participants were required to consume one smoothie each day under a supervision of a clinical coordinator.

6.2.3 Physical activity assessment

Physical activity was monitored using Actigraph GT3X+ activity monitors. The monitor was worn at the waist and recorded the activity for seven days (days 29-35) of each phase.

Accelerometer is a validated advanced technology that provides a trustworthy measurement of physical activity and is widely used in physical activity research (2, 3). ActiLife 6 analysis software (Pensacola, FL, USA) was programmed with the participant code, height, weight, gender, race and date of birth. To reduce the instrument variation, the serial number of the monitor used for each participant was recorded and each participant was given the same monitor throughout the study. The activity was assessed as a composite vector magnitude of the three axes of the activity monitor and reported as counts/min/d (cpm) (7). Here, we were interested in the spontaneous physical activity which defined as energy expenditure resulting primarily from unstructured mobility-related activities that occur during daily life.

6.2.4 Statistical analyses

Statistical analyses were performed using SAS 9.4 (SAS, Cary, NC) based on a per protocol approach. Normality was assessed using the Shapiro–Wilk test and the skewness value. Non-normally distributed variables were log-transformed before analysis. The results are expressed as least square means ± SEMs unless otherwise specified. Statistical significance was set at P-value < 0.05. PROC MIXED with repeated-measure procedure was used to assess the effect of the three dietary treatments on physical activity level. Treatment, sex, age, and genotype were used as fixed effects, with participants as a repeated factor. Random effects were identified as treatment sequence, clinical site, and participants.

6.3 Results and Discussion

Fifty subjects participated in this analysis; however, 31 participants complete the analysis for the three phases and were included in the statistical analyses. Participant characteristics are

displayed in **Table 6.1**. This study found no difference across the three treatments in physical activity level **Table 6.2**. All of the participants had light physical activity levels across the three treatments, according to the cutoff point for moderate activity (2690 cpm) reported till date (8).There was no difference observed between male and female. Metabolic equivalent (MET) did not influence by the treatment intervention and all participants fall into the light MET category values (9).

Table 6.1: Characteristics of participants at baseline of the dietary intervention[1]

Characteristic	Total n=31	Male n=10	Female n=21
Age	43.07 ± 2.83	37.111 ± 3.069	46.056 ± 2.33*
Waist circumference (cm)	105.08 ± 3.28	111.178 ± 5.576	102.02 ± 2.13*
Weight (kg)	91.49 ± 4.77	106.43 ± 7.00	84.03 ± 2.89*
BMI[3] (kg/m^2)	32.27 ± 1.38	33.45 ± 2.57	31.67 ± 0.88
Systolic BP (mmHg)	121.0 ± 2.9	125.4 ± 3.8	118.8 ± 2.4
Diastolic BP (mmHg)	82.54 ± 2.22	87.11 ± 2.88	80.12 ± 1.72
Cholesterol (mmol/L)	5.42 ± 0.23	5.56 ± 0.38	5.34 ± 0.17
Triglycerides (mmol/L)	1.90 ± 0.20	2.09 ± 0.30	1.81 ± 0.16
HDL-cholesterol (mmol/L)	1.26 ± 0.09	0.98 ± 0.05	1.39 ± 0.07*
LDL-cholesterol (mmol/L)	3.29 ± 0.20	3.62 ± 0.33	3.13 ± 0.14
Glucose (mg/dl)	96.25 ± 2.30	94.45 ± 2.63	97.13 ± 2.02
Insulin (μIU/ml)	18.37 ± 2.76	24.17 ± 5.44	15.46 ± 1.31*

[1]All values are means ± SEMs. SAS PROC MIXED procedure used to assess the inter-sex differences, $P<0.05$ was considered significant. SAS PROC MEANS used to determine the mean characteristics of the overall population. * Indicates significant difference between male and female.
[2]BP: blood pressure; BMI: body mass index; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

Table 6.2: Physical activity measures according to dietary intervention[1]

Variable	Treatment	Total n=31	Male n=10	Female n=21
Counts/min/d	HOCO	368.6 ± 1.1	342.8 ± 1.1	396.5 ± 1.1
	RCO	357.8 ± 1.1	324.2 ± 1.1	395.1 ±1.1
	Control	378.4 ± 1.1	354.9 ± 1.1	403.5 ± 1.1
Metabolic equivalent	HOCO	1.137 ± 0.031	1.162 ± 0.041	1.113 ± 0.033
	RCO	1.135 ± 0.031	1.161 ± 0.041	1.108 ± 0.033
	Control	1.138 ± 0.031	1.165 ± 0.041	1.110 ± 0.033

[1]All data represent the endpoint measures and are presented as least-squares means ± SEMs. Control: low-MUFA high-SFA treatment; HOCO: high-oleic canola; MUFA: monounsaturated fatty acids; RCO: regular canola oil. SAS PROC MIXED with repeated measure procedure used to assess the effect of MUFA consumption on physical activity, using participants' as a repeated factor. Treatment, sex, age, and genotype were used as fixed effects. Random effects were treatment sequence, clinical site, and participants. $P<0.05$ was considered significant. No significant differences were observed in the overall population or between male and female

Kien at al. have suggested a favorable effect of a high-MUFA diet on cognition that could influence the behavioral choices toward more active status compared to a high-SFA diet (7). This trial cannot reject the possible beneficial effect of high-MUFA consumption on physical activity level proposed by aforementioned study due to several reasons. First, characteristics of each study participants; subjects of the previous study were healthy young adults and mostly nonobese; however, here, all participants had abdominal obesity plus at least one metabolic syndrome risk factor. Such variations, especially obesity, might reflect a sedentary lifestyle as well as behavioral choices that might possibly have contributed to a resistance to elevate the

spontaneous level of physical activity (9, 10). Additionally, the women-to-men ratio was one and 2.1 in Kien et al. and this study, respectively. Variation in sex distribution across the two trials might have led to conflicting findings (11, 12).

Secondly, the percent of carbohydrate and fat as a function of total energy as well as fatty acid proportions across the two trials are different, thereby, render it difficult to discern which findings are more conclusive regarding the effect of elevated MUFA consumption on physical activity level, especially given that the evidence is still lacking on the optimal dietary fatty acid proportion as well as due to the variations in metabolism and substrate availability of macronutrients (13). Further, COMIT II was designed to ensure iso-energic conditions, in order to better estimate the influence of MUFA consumption on body weight and fat mass changes. Therefore, we consistently reminded our participants to maintain their physical activity level throughout the study, which might have hindered the proposed effect of MUFA consumption on physical activity level.

Studies have suggested a wide range of heritability estimates that might explain up to 60% of physical activity levels (14-16). Although the causal relationships between physical activity and genetics have not been well-established, a few strong candidate genes have been identified thus far (14, 17). Therefore, the propensity toward being spontaneously active could be partly influenced by genotypes. Nevertheless, here, assessing the influence of genetic architecture on physical activity level in such a small sample size (n=31) will not generate a sufficiently reliable prediction of any possible association (18). In the same context, physical activity level might modulate the effect of genetic polymorphisms on body weight (19-22), which might have contributed to the null overall finding of this trial on obesity discussed in Chapter III.

Fundamentally, obesity is caused by a disruption of energy balance. With the burgeoning prevalence of obesity and sedentary lifestyle worldwide, dietary fatty acid manipulation might hold a promise toward less intensive but effective weight control strategy. Further research is definitely warranted to draw a conclusion on whether or not high-MUFA consumption can elevate physical activity level.

6.4 References

1. Fox KR, Hillsdon M. Physical activity and obesity. Obes Rev 2007;8 Suppl 1:115-21. doi: 10.1111/j.1467-789X.2007.00329.x.

2. Bisschop CN, Peeters PH, Monninkhof EM, van der Schouw YT, May AM. Associations of visceral fat, physical activity and muscle strength with the metabolic syndrome. Maturitas 2013;76(2):139-45. doi: 10.1016/j.maturitas.2013.06.015.

3. Murabito JM, Pedley A, Massaro JM, Vasan RS, Esliger D, Blease SJ, Hoffman U, Fox CS. Moderate-to-vigorous physical activity with accelerometry is associated with visceral adipose tissue in adults. J Am Heart Assoc 2015;4(3):e001379. doi: 10.1161/jaha.114.001379.

4. Manson JE, Skerrett PJ, Greenland P, VanItallie TB. The escalating pandemics of obesity and sedentary lifestyle. A call to action for clinicians. Arch Intern Med 2004;164(3):249-58. doi: 10.1001/archinte.164.3.249.

5. Krishnan S, Cooper JA. Effect of dietary fatty acid composition on substrate utilization and body weight maintenance in humans. Eur J Nutr 2014;53(3):691-710. doi: 10.1007/s00394-013-0638-z.

6. Kien CL, Bunn JY. Gender alters the effects of palmitate and oleate on fat oxidation and energy expenditure. Obesity (Silver Spring) 2008;16(1):29-33. doi: 10.1038/oby.2007.13.

7. Kien CL, Bunn JY, Tompkins CL, Dumas JA, Crain KI, Ebenstein DB, Koves TR, Muoio DM. Substituting dietary monounsaturated fat for saturated fat is associated with increased daily physical activity and resting energy expenditure and with changes in mood. Am J Clin Nutr 2013;97(4):689-97. doi: 10.3945/ajcn.112.051730.

8. Sasaki JE, John D, Freedson PS. Validation and comparison of ActiGraph activity monitors. J Sci Med Sport 2011;14(5):411-6. doi: 10.1016/j.jsams.2011.04.003.

9. Levine JA, Lanningham-Foster LM, McCrady SK, Krizan AC, Olson LR, Kane PH, Jensen MD, Clark MM. Interindividual variation in posture allocation: possible role in human obesity. Science 2005;307(5709):584-6. doi: 10.1126/science.1106561.

10. Katzmarzyk PT, Lee IM. Sedentary behaviour and life expectancy in the USA: a cause-deleted life table analysis. BMJ Open 2012;2(4). doi: 10.1136/bmjopen-2012-000828.

11. Lin YC, Yeh MC, Chen YM, Huang LH. Physical activity status and gender differences in community-dwelling older adults with chronic diseases. J Nurs Res 2010;18(2):88-97. doi: 10.1097/JNR.0b013e3181dda6d8.

12. Azevedo MR, Araújo CLP, Reichert FF, Siqueira FV, da Silva MC, Hallal PC. Gender differences in leisure-time physical activity. Edtion ed. Int J Public Health. Basel, 2007:8-15.

13. Jeukendrup AE. Modulation of carbohydrate and fat utilization by diet, exercise and environment. Biochem Soc Trans 2003;31(Pt 6):1270-3. doi: 10.1042/.

14. Moore-Harrison T. Driven to Be Inactive?—The Genetics of physical activity. 2010;94:271-90. doi: 10.1016/b978-0-12-375003-7.00010-8.

15. Maia JA, Thomis M, Beunen G. Genetic factors in physical activity levels: a twin study. Am J Prev Med 2002;23(2 Suppl):87-91.

16. Stubbe JH, Boomsma DI, Vink JM, Cornes BK, Martin NG, Skytthe A, Kyvik KO, Rose RJ, Kujala UM, Kaprio J, et al. Genetic influences on exercise participation in 37.051 twin pairs from seven countries. Edtion ed. In: Feldman M, ed. PLoS One. San Francisco, USA, 2006.

17. Lightfoot JT. Current understanding of the genetic basis for physical activity. J Nutr 2011;141(3):526-30. doi: 10.3945/jn.110.127290.

18. Hong EP, Park JW. Sample size and statistical power calculation in genetic association studies. Genomics Inf 2012;10(2):117-22. doi: 10.5808/gi.2012.10.2.117.

19. Andreasen CH, Stender-Petersen KL, Mogensen MS, Torekov SS, Wegner L, Andersen G, Nielsen AL, Albrechtsen A, Borch-Johnsen K, Rasmussen SS, et al. Low physical activity accentuates the effect of the FTO rs9939609 polymorphism on body fat accumulation. Diabetes 2008;57(1):95-101. doi: 10.2337/db07-0910.

20. Rampersaud E, Mitchell BD, Pollin TI, Fu M, Shen H, O'Connell JR, Ducharme JL, Hines S, Sack P, Naglieri R, et al. Physical activity and the association of common FTO gene variants with body mass index and obesity. Arch Intern Med 2008;168(16):1791-7. doi: 10.1001/archinte.168.16.1791.

21. Corbalan MS, Marti A, Forga L, Martinez-Gonzalez MA, Martinez JA. The 27Glu polymorphism of the beta2-adrenergic receptor gene interacts with physical activity influencing obesity risk among female subjects. Edtion ed. Clin Genet. Denmark, 2002:305-7.

22. Otabe S, Clement K, Dina C, Pelloux V, Guy-Grand B, Froguel P, Vasseur F. A genetic variation in the 5' flanking region of the UCP3 gene is associated with body mass index in humans in interaction with physical activity. Diabetologia 2000;43(2):245-9. doi: 10.1007/s001250050037.

CHAPTER VII

GENERAL DISCUSSION

7.1 Summary and implications

The findings of the studies incorporated in this thesis contribute to the existing knowledge concerning interactions between genes and dietary fatty acid (FA) composition and essentially emphasize the benefit of personalized nutrition in modulating health outcomes. Fatty acid composition has a strong influence on obesity and health abnormalities (1, 2). With the increased demand on reducing saturated FA consumption and taking into account the debate regarding the effect of n-6 polyunsaturated FA on metabolic health (3, 4), we evaluated as whether monounsaturated FA (MUFA) might be the best candidate to replace saturated fat. Although the evidence underscores the advantageous influence of MUFA consumption on health outcomes, it would be of great benefit to elucidate the specific effects of MUFA on body fat distribution which enhances our understanding of obesity-associated metabolic abnormalities. For instance, strong relationships exist between abdominal obesity and several cardiovascular disease risk factors, where a three-fold increase in cardiovascular disease risk factors corresponded to an increase of less than 1 kg of abdominal fat (5). Using surrogate biomarkers to assess obesity and fat distribution, such as body mass index and WHR, may hinder the ability to demonstrate the possible substantial effect of FA modification on fat distribution and, therefore, metabolic abnormality, consequently, critical assessment of fat mass and distribution is essential for future research. Herein, body composition was assessed using whole-body dual X-rays scanning which provides a reliable identification of fat distribution and discrimination between different fat depots. However, in the sample as a whole, our results were inconsistent with the

growing evidence that suggests favorable effects of MUFA consumption on body weight (6, 7), fat distribution (8, 9), and insulin resistance (IR) (10, 11). Perhaps the fact that our participants had abdominal obesity plus at least one metabolic syndrome risk factors, including elevated glucose concentration, might have mitigated the beneficial effects of MUFA consumption in an iso-caloric scenario in the overall population. Several adipokines that fundamentally secreted from adipose tissue in the abdominal area might have influenced lipid and glucose metabolism and contributed to the null effects of MUFA on body weight, body fat, and IR (12, 13). Another suggested reason is that the elevated baseline fasting plasma glucose concentration, possibly reflecting IR, might have reduced the effectiveness of our dietary intervention, as indicated by a recent data analysis from three randomized trials of overweight and obese participants where baseline fasting concentrations of glucose and insulin modulated the response to dietary interventions (14). Additionally, the present research confers another explanation to the null effect of MUFA consumption by illuminating the inter-individual heterogeneity in the responsiveness of body fat mass and IR measure to dietary MUFA. Therefore, inter-population genetic variations may explain our inability to detect significant effects of MUFA consumption on body weight, fat mass, and IR in spite of the existing evidence.

Single nucleotide polymorphisms (SNPs) within *LPL*, *FTO*, *PPARα*, *ADIPOQ*, *APOE*, *LIPC*, and *ADRB2* genes modulated body fat mass distribution in response to different levels of dietary MUFA in an iso-caloric diet. The most important finding was the capacity of *LPL* rs13702-CC genotype to reduce VAT, android fat mass, trunk fat mass, total fat mass, and body weight following high-MUFA consumption compared to the low-MUFA diet. The fact that the response of different regions of fat mass to the high-MUFA diets in an iso-caloric condition was modulated by this genotype provides validity to this interaction. Although the observed

modulatory effects of the seven SNPs on changes in total and compartmental fat mass in response to various levels of dietary MUFA were small over six weeks, their statistical significance may indicate a potential clinical effect in weight reduction/maintenance regimens over prolonged periods. Also, detecting these changes in body fat regardless of the controlled iso-caloric scenario may provides certainty to the influence of dietary FA on pathways controlled by these genes. These findings may eventually contribute to the development of predictive tools for administering optimal and personalized weight management interventions based on an individual's genetic makeup.

Exploring the effect of MUFA on gene expression levels aids in illuminating the underlying mechanisms of the beneficial effects of MUFA consumption on energy balance that has been previously reported in the literature. MUFA consumption was found to modulate the relative expression of *CPT1B* and to influence the relationship between expression levels of *PLIN1* and *SCD1* and regional body fat. From our study, we could not reach a definite conclusion as we did not measure enzyme/protein activities, thus, we encourage future studies that target expression and activity of more enzymes involved in fat oxidation and obesogenic pathways.

Chapter V discusses the association between genetic architecture and dietary FA modification on IR and glycemic control measures. Although the number of SNPs that have been identified, thus far, to be associated with IR is limited, the influence of genetic predisposition on modulating the response of IR measure to dietary FA modification has been previously reported (15-18). In this project, dietary FA composition modified IR measures in the carriers of either the *FABP1* rs2241883-CC or *IRS1* rs7578326-GG genotype compared to the carriers of the opposite genotypes within the corresponding gene. The carriers of the aforementioned genotypes had

lower HOMA-IR levels following the low-MUFA high-SFA treatment, which may suggest protective effects of these genotypes against the deleterious effect of elevated SFA consumption on IR. This finding supports the promising concept of personalized nutrition which may eventually contribute to developing an effective genotype-based dietary recommendation to reduce IR incidence and complications.

7.2 Strengths, limitations and future directions

The completed trial represented a significant success which can be attributed to the dedication and commitment of all study participants, investigators, clinical coordinators, and supporting staff. The strengths of the study were controlled full-feeding and the crossover design with relatively large sample size for such trial. The controlled full-feeding scenario is the "gold standard" for assessing the health benefits of dietary interventions. The full-feeding eliminated the effects of variable background diets on study outcomes and the crossover design minimized the effect of individual differences in response to treatments which significantly reduced the required sample size.

Although this population size is larger than most similar dietary interventions and detected/replicated some gene-diet interactions, it is still considered small in the context of examining genetic factors and might not be sufficient to offer definite conclusions. In this context, unevenly distributed frequencies of genetic variants within the SNPs under study were often observed. Therefore, the findings of novel associations in this research would need replication in trials of larger sizes to overcome this shortcoming. Another point relates to the

limitations could be the subgroup analysis of gene expression on body fat mass. However, this was a secondary exploratory analysis which would direct future investigations.

Further, considering that we took a candidate gene approach, only a limited number of systematically-chosen SNPs within certain genes associated with obesity and IR were studied in the present research. Even though many SNPs have been identified, to date, to contribute to the inter-individual variability in the response of body weight to dietary fatty acid modification, the explained variability of obesity by these loci remains low. Many other SNPs that are associated with obesity and IR are yet to be analyzed, in future investigations, for their interactions with fatty acid composition. Of note, however, failure to detect an association with various selected SNPs in this study does not exclude the possibility that other SNPs in the candidate genes are related to fat loss following MUFA consumption. Future studies can defeat this pitfall by using next-generation DNA sequencing to thoroughly analyze the entire genes of interest for associations which also would eliminate bias in the selection of genes and SNPs of interest (19-21).

Additionally, in context with the limitation above, we did not evaluate the effect of combinations of SNPs on obesity due to the difficulty to perform this evaluation on 21 SNPs manually. The analysis of the effect of separate SNP is a limitation of this, as well as most of the available, research which might contribute to the limited ability to explain inter-individual variability in body weight response. Yet, obesity is a complex trait, which is likely to involve numerous genes implicated in controlling adipogenesis, lipid turnover, appetite regulation, energy balance, as well as lipid metabolism, hence, the logical assumption would be that a significant part of its variability is explained by the additive effect of several SNPs which, taken separately, may have a negligible significant effect. Thus, to reach the ultimate aim of studying

gene-diet interactions, which is personalized dietary intervention, the future investigations must focus on evaluating the influence of combinations of SNPs. This also true for associating gene-diet interaction on IR and other health conditions. The analysis of combinations of a large number of SNP, particularly if used along with genome-wide analysis, requires sophisticated technology for reliable evaluation such as machine learning technology (22).

One important limitation of this study is that we did not apply stringent control for multiple testing, which may lead to a potential overstatement of our findings (i.e. type I, false positive) error. Considering these limitations, future dietary intervention studies need to prevail over these limitations to enable a thorough understanding of genetic heterogeneity in obesity and IR responsiveness to dietary MUFA. Further, a priori selection and recruitment of a large sample size of carriers of genotypes known to be associated with hyper- and hypo-responsiveness to dietary MUFA can be adopted to validate the current findings and enhance our knowledge on the effect of MUFA consumption on obesity and its health consequences.

7.3 Final conclusion

The prevalence of obesity and IR is skyrocketing globally despite the astonishing efforts invested by healthcare researchers to control their occurrence (23, 24). Obesity is a leading cause of morbidity and mortality and is strongly and independently associated with IR which, in turn, further augments disease and obesity risk (25). Modulation dietary FA composition has been identified as a strong influencer of obesity and IR risk, with an increased support of the beneficial leverage of MUFA consumption on these health outcomes (7, 8, 26, 27). However, given the well-identified involvement of genetics in the development of obesity and IR, the

responsiveness of these health outcomes to dietary MUFA is profoundly determined by inter-individual heterogeneity. Hyper- and hypo-responsiveness of body fat changes and IR measures to dietary MUFA have been previously reported in the literature (28-30). The work presented in this thesis supports the importance of diet-gene interactions in modulating health status.

By using a reliable identification of body fat mass and distribution, this thesis highlights an important contribution of genetic predisposition in the response of total and regional fat mass to dietary FA modification, which might be hindered in previous research due to the use of surrogate measures of obesity. The observations encompassed in this thesis might explain the inconsistency in the available evidence regarding the effect size of dietary MUFA on obesity and IR, as well as might explain the general low success rate of obesity control regimens. The results of this project reinforce the need for personalized intervention which can improve health and wellness and promotes better compliance rate to dietary and lifestyle intervention. Furthermore, the association between some polymorphisms and particular regions of body fat in response to different levels of dietary MUFA that observed in this thesis might reflect inter-depot variation in response to dietary fatty acid modification. Such findings encourage the importance of accurate assessment of regional body fat mass and the discrimination between fat depots which will lead to an advanced understanding of the pathogenicity of obesity and to a superior weight control intervention. Lastly, obesity is a complex trait with three predominant contributors including diet, lifestyle and genetic. Therefore, ultimately, linking the nutrient intake and lifestyle with the genetic makeup would provide a sophisticated realization of the health impact of dietary factors, including fatty acid composition, and will lead to optimal health and disease prevention.

7.4 References

1. Lovejoy JC. The influence of dietary fat on insulin resistance. Curr Diab Rep 2002;2(5):435-40.

2. Krishnan S, Cooper JA. Effect of dietary fatty acid composition on substrate utilization and body weight maintenance in humans. Eur J Nutr 2014;53(3):691-710. doi: 10.1007/s00394-013-0638-z.

3. Russo GL. Dietary n-6 and n-3 polyunsaturated fatty acids: from biochemistry to clinical implications in cardiovascular prevention. Biochem Pharmacol 2009;77(6):937-46. doi: 10.1016/j.bcp.2008.10.020.

4. Simopoulos AP. An increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. Nutrients 2016;8(3):128. doi: 10.3390/nu8030128.

5. Wiklund P, Toss F, Weinehall L, Hallmans G, Franks PW, Nordstrom A, Nordstrom P. Abdominal and gynoid fat mass are associated with cardiovascular risk factors in men and women. J Clin Endocrinol Metab 2008;93(11):4360-6. doi: 10.1210/jc.2008-0804.

6. Mendez MA, Popkin BM, Jakszyn P, Berenguer A, Tormo MJ, Sanchez MJ, Quiros JR, Pera G, Navarro C, Martinez C, et al. Adherence to a Mediterranean diet is associated with reduced 3-year incidence of obesity. J Nutr 2006;136(11):2934-8.

7. Alves RD, Moreira AP, Macedo VS, de Cassia Goncalves Alfenas R, Bressan J, Mattes R, Costa NM. Regular intake of high-oleic peanuts improves fat oxidation and body composition in overweight/obese men pursuing a energy-restricted diet. Obesity (Silver Spring) 2014;22(6):1422-9. doi: 10.1002/oby.20746.

8. Liu X, Kris-Etherton PM, West SG, Lamarche B, Jenkins DJ, Fleming JA, McCrea CE, Pu S, Couture P, Connelly PW, et al. Effects of canola and high-oleic-acid canola oils on abdominal fat mass in individuals with central obesity. Obesity (Silver Spring) 2016;24(11):2261-8. doi: 10.1002/oby.21584.

9. Silver HJ, Kang H, Keil CD, Muldowney JA, 3rd, Kocalis H, Fazio S, Vaughan DE, Niswender KD. Consuming a balanced high fat diet for 16 weeks improves body composition, inflammation and vascular function parameters in obese premenopausal women. Metabolism 2014;63(4):562-73. doi: 10.1016/j.metabol.2014.01.004.

10. Paniagua JA, Gallego de la Sacristana A, Romero I, Vidal-Puig A, Latre JM, Sanchez E, Perez-Martinez P, Lopez-Miranda J, Perez-Jimenez F. Monounsaturated fat-rich diet prevents central body fat distribution and decreases postprandial adiponectin expression induced by a carbohydrate-rich diet in insulin-resistant subjects. Diabetes Care 2007;30(7):1717-23. doi: 10.2337/dc06-2220.

11. Vessby B, Uusitupa M, Hermansen K, Riccardi G, Rivellese AA, Tapsell LC, Nalsen C, Berglund L, Louheranta A, Rasmussen BM, et al. Substituting dietary saturated for

monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. Diabetologia 2001;44(3):312-9.

12. Rambhojan C, Bouaziz-Amar E, Larifla L, Deloumeaux J, Clepier J, Plumasseau J, Lacorte JM, Foucan L. Ghrelin, adipokines, metabolic factors in relation with weight status in school-children and results of a 1-year lifestyle intervention program. Nutr Metab (Lond) 2015;12:43. doi: 10.1186/s12986-015-0039-9.

13. Kimura Y, Pham NM, Yasuda K, Nanri A, Kurotani K, Kuwahara K, Akter S, Sato M, Hayabuchi H, Mizoue T. Association of adulthood weight gain with circulating adipokine and insulin resistance in the Japanese population. Eur J Clin Nutr 2015;69(4):462-6. doi: 10.1038/ejcn.2014.257.

14. Hjorth MF, Ritz C, Blaak EE, Saris WH, Langin D, Poulsen SK, Larsen TM, Sorensen TI, Zohar Y, Astrup A. Pretreatment fasting plasma glucose and insulin modify dietary weight loss success: results from 3 randomized clinical trials. Am J Clin Nutr 2017;106(2):499-505. doi: 10.3945/ajcn.117.155200.

15. Zheng Y, Huang T, Zhang X, Rood J, Bray GA, Sacks FM, Qi L. Dietary fat modifies the effects of FTO genotype on changes in insulin sensitivity. J Nutr 2015;145(5):977-82. doi: 10.3945/jn.115.210005.

16. Zheng JS, Arnett DK, Parnell LD, Smith CE, Li D, Borecki IB, Tucker KL, Ordovas JM, Lai CQ. Modulation by dietary fat and carbohydrate of IRS1 association with type 2 diabetes traits in two populations of different ancestries. Diabetes Care 2013;36(9):2621-7. doi: 10.2337/dc12-2607.

17. Smith CE, Arnett DK, Corella D, Tsai MY, Lai CQ, Parnell LD, Lee YC, Ordovas JM. Perilipin polymorphism interacts with saturated fat and carbohydrates to modulate insulin resistance. Nutr Metab Cardiovasc Dis 2012;22(5):449-55. doi: 10.1016/j.numecd.2010.09.003.

18. Luan J, Browne PO, Harding AH, Halsall DJ, O'Rahilly S, Chatterjee VK, Wareham NJ. Evidence for gene-nutrient interaction at the PPARgamma locus. Diabetes 2001;50(3):686-9.

19. Shendure J. Next-generation human genetics. Edtion ed. Genome Biol. England, 2011:408.

20. Shendure J, Ji H. Next-generation DNA sequencing. Nat Biotechnol 2008;26(10):1135-45. doi: 10.1038/nbt1486.

21. ten Bosch JR, Grody WW. Keeping up with the next generation: massively parallel sequencing in clinical diagnostics. J Mol Diagn 2008;10(6):484-92. doi: 10.2353/jmoldx.2008.080027.

22. Libbrecht MW, Noble WS. Machine learning applications in genetics and genomics. Nat Rev Genet 2015;16(6):321-32. doi: 10.1038/nrg3920.

23. Bancej C, Jayabalasingham B, Wall RW, Rao DP, Do MT, de Groh M, Jayaraman GC. Trends and projections of obesity among Canadians. Edtion ed. Health Promot Chronic Dis Prev Can, 2015:109-12.

24. Bermudez V, Salazar J, Martinez MS, Chavez-Castillo M, Olivar LC, Calvo MJ, Palmar J, Bautista J, Ramos E, Cabrera M, et al. Prevalence and associated factors of insulin resistance in adults from Maracaibo City, Venezuela. Adv Prev Med 2016;2016:9405105. doi: 10.1155/2016/9405105.

25. Martinez KE, Tucker LA, Bailey BW, LeCheminant JD. Expanded normal weight obesity and insulin resistance in US adults of the National Health and Nutrition Examination Survey. J Diabetes Res 2017;2017:9502643. doi: 10.1155/2017/9502643.

26. Alvarez-Perez J, Sanchez-Villegas A, Diaz-Benitez EM, Ruano-Rodriguez C, Corella D, Martinez-Gonzalez MA, Estruch R, Salas-Salvado J, Serra-Majem L. Influence of a Mediterranean dietary pattern on body fat distribution: results of the PREDIMED-Canarias Intervention Randomized Trial. J Am Coll Nutr 2016;35(6):568-80. doi: 10.1080/07315724.2015.1102102.

27. Gillingham LG, Robinson KS, Jones PJ. Effect of high-oleic canola and flaxseed oils on energy expenditure and body composition in hypercholesterolemic subjects. Metabolism 2012;61(11):1598-605. doi: 10.1016/j.metabol.2012.04.016.

28. Razquin C, Martinez JA, Martinez-Gonzalez MA, Fernandez-Crehuet J, Santos JM, Marti A. A Mediterranean diet rich in virgin olive oil may reverse the effects of the -174G/C IL6 gene variant on 3-year body weight change. Mol Nutr Food Res 2010;54 Suppl 1:S75-82. doi: 10.1002/mnfr.200900257.

29. Razquin C, Martinez JA, Martinez-Gonzalez MA, Bes-Rastrollo M, Fernandez-Crehuet J, Marti A. A 3-year intervention with a Mediterranean diet modified the association between the rs9939609 gene variant in FTO and body weight changes. Int J Obes (Lond) 2010;34(2):266-72. doi: 10.1038/ijo.2009.233.

30. Corella D, Carrasco P, Sorli JV, Estruch R, Rico-Sanz J, Martinez-Gonzalez MA, Salas-Salvado J, Covas MI, Coltell O, Aros F, et al. Mediterranean diet reduces the adverse effect of the TCF7L2-rs7903146 polymorphism on cardiovascular risk factors and stroke incidence: a randomized controlled trial in a high-cardiovascular-risk population. Diabetes Care 2013;36(11):3803-11. doi: 10.2337/dc13-0955.

APPENDICES

APPENDIX I

Ethics Approval for Studies Corresponding to Chapters III, IV, V, VI

UNIVERSITY OF MANITOBA | BANNATYNE CAMPUS
Research Ethics Boards

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BIOMEDICAL RESEARCH ETHICS BOARD (BREB)
CERTIFICATE OF FINAL APPROVAL FOR NEW STUDIES
Full Board Review

PRINCIPAL INVESTIGATOR:	INSTITUTION/DEPARTMENT:	ETHICS #:
Dr. P. Jones	U of M/RCFFN	B2013:137
BREB MEETING DATE:	**APPROVAL DATE:**	**EXPIRY DATE:**
September 23, 2013	October 10, 2013	September 23, 2014
STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (If applicable):		

PROTOCOL NUMBER:	PROJECT OR PROTOCOL TITLE:
	Effects of Canola Oil on Body Composition and Lipid Metabolism in Participants with Metabolic Syndrome/Canola Oil Multi-Center Intervention Trial 2 (COMIT II) (Linked to B2010:052 and B2007:071)
SPONSORING AGENCIES AND/OR COORDINATING GROUPS: Canola Council of Canada, DOW Agrosciences and Agriculture and Agri-Food Canada	

Submission Date(s) of Investigator Documents:	REB Receipt Date(s) of Documents:
September 3 and October 1, 2013	September 3 and October 7, 2013

THE FOLLOWING ARE APPROVED FOR USE:

Document Name	Version(if applicable)	Date
Protocol:		
Protocol		September 3, 2013
Consent and Assent Form(s):		
Research Subject Information and Consent Form	V. 2	October 1, 2013
RCFFN Preliminary Trial Screening Consent		September 3, 2013
Additonal Research Subject Information and Consent Form for Genetic Analysis	V. 1	September 3, 2013
Other:		
Poster 1 and 2	V. 1	September 3, 2013
Participant Screening Form		
General Information Sheet	V. 1	September 3, 2013

CERTIFICATION
The University of Manitoba (UM) Biomedical Research Board (BREB) has reviewed the research study/project named on this ***Certificate of Final Approval*** at the ***full board meeting*** date noted above and was found to be acceptable on ethical grounds for research involving human participants. The study/project and documents listed above was granted final approval by the Chair or Acting Chair, UM BREB.

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 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,

[redacted]

Lindsay Nicolle, 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 I: Ethics Approval for Studies Corresponding to Chapters III, IV, V, VI

UNIVERSITY OF MANITOBA | Research Ethics - Bannatyne
Office of the Vice-President (Research and International)

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

BIOMEDICAL RESEARCH ETHICS BOARD (BREB)

CERTIFICATE OF ANNUAL APPROVAL

PRINCIPAL INVESTIGATOR:	INSTITUTION/DEPARTMENT:	ETHICS #:
Dr. Peter Jones	U of M and RCFFN/Food Sciences	HS18154 (B2013:137)
BREB MEETING DATE (If applicable):	**APPROVAL DATE:** September 11, 2017	**EXPIRY DATE:** **September 23, 2018**
STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (If applicable):		

PROTOCOL NUMBER:	PROJECT OR PROTOCOL TITLE:
NA	Canola Oil Multi-center Intervention Trial II (COMIT II): Effects of Oleic Acid Enriched and Regular Canola Oil on Body Composition and Lipid Metabolism in Participants with Metabolic Syndrome Risk Factors (Linked to B2010:052 and B2007:071)
SPONSORING AGENCIES AND/OR COORDINATING GROUPS: Canola Council of Canada, DOW Agrosciences and Agriculture and Agri-Foods Canada	

Submission Date of Investigator Documents:	BREB Receipt Date of Documents:
August 1, 2017	August 4, 2017

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

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,

[redacted]

Lindsay Nicolle, 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

Forms Corresponding to Studies Described in Chapters III, IV, V, VI

Study Advertisements – Poster

UNIVERSITY OF MANITOBA

Richardson Centre for Functional Foods and Nutraceuticals

Do you have extra belly fat?

The Richardson Centre for Functional Foods and Nutraceuticals is conducting a nutrition study to investigate how dietary oils can improve your health while potentially reducing android (belly) fat.

The study is open to men and women who meet the following criteria:

- Aged 20-65
- Elevated waist circumference
- Not taking medication to lower blood lipids/cholesterol
- Non-smoker

Volunteers will be compensated for their participation.

Phone: (204) 480-1042

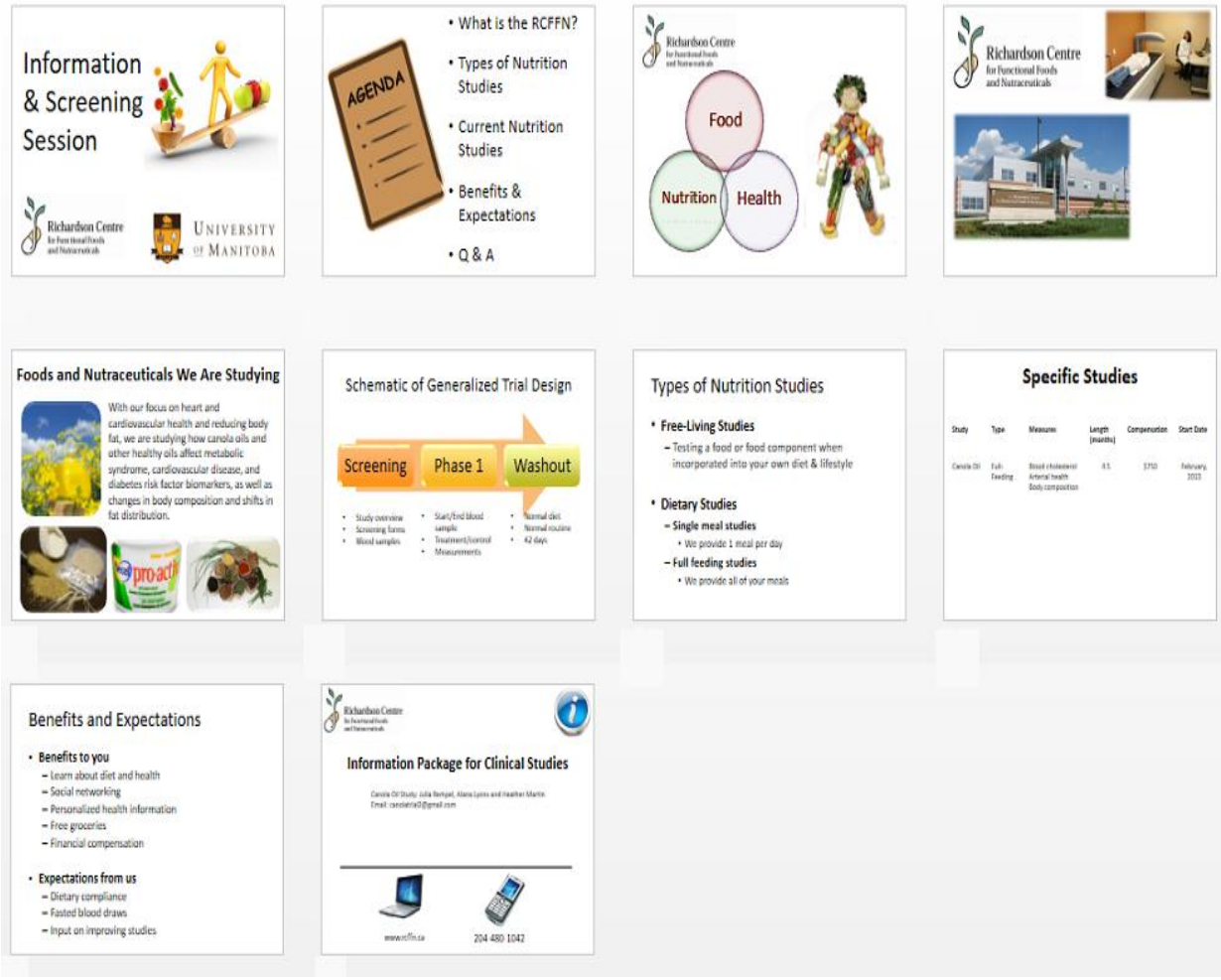
Email: canola.trial@umanitoba.ca

Website: www.rcffn.ca

Dr. Peter Jones, Principal Investigator

Appendix II

Study Information Session Presentation



Appendix II

Subject Screening Consent Form

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

RCFFN preliminary trial screening consent

You have expressed an interest in participating in a study at the Richardson Center for Functional Foods and Nutraceuticals. You have been invited to have your health assessed to determine if you meet the requirements of the study.

The clinical coordinator team will assess your cholesterol level, medical history, body measurements, and your availability over the next 2 years. Depending on your results you will be offered the opportunity to participate in a study.

To allow the necessary information to be obtained, you agree to provide fasting blood samples (approximately 10 ml or 2 teaspoons) for the measurement of blood cholesterol, blood count, and iron levels.

Prior to taking part in any study, you will be given the specific study consent form to read and sign if you are still interested in participating.

The blood is taken from a vein in the forearm, as is usually done during a blood test. 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.

I understand I can withdraw from this process at any time at my discretion.

Participant's Signature	Participant's Name (please print)	Date
Investigator's Signature **(or Clinical Coordinator)**	Investigator's Name (please print) **(position**	Date

Appendix II

Medical Examination Form

Medical Examination Form

Phase Pre-study	**Study Physician** Dr.	
Date of Visit ____/____/____ MM DD YR	**Investigator** Dr. Peter Jones	**Subject Code**

COMPLETE PHYSICAL EXAMINATION

A. Vital Signs

Body Weight:_____ lbs _____ kg **Height**: _____cm

Respirations:_____

Blood Pressure (seated): _____/_____ mmHg **Heart Rate**:_____bpm
systolic diastolic

Race/Ethnic Origin:
□ **Caucasian** □ **African-American/Canadian** □ **Asian** □
Other:_____

B. Body Systems (Check the appropriate box if organ system was examined. If not done, write N/D in the box)

	Normal	Abnormal	*Details of abnormal finding
1) Ears, Nose, Throat			
2) Eyes			
3) Dermatological			
4) Musculoskeletal			
5) Lymph Nodes			
6) Neurological			
7) Cardiovascular			
8) Respiratory			
9) Endocrine			
10) Urogenital			

11) Gastrointestinal (complete section C)			

C. Gastrointestinal Cont…

Bowel Habits: Frequency_____/Day **Urination:** Frequency_____/Day

Consistency_____
Nocturia_____/Night

Medications:

Hospitalizations:

Family History:

D. Medical History

	YES	**NO**
Have you taken a glucose lowering medication or a medication affecting lipid metabolism (cholestyramine, colestipol, niacin, colfibrate, gemfibrozil, probucol, HMG-CoA reductase inhibitors, and high-dose dietary supplements, plant sterols or fish oil capsules) within the past 3 months?		
Do you take systemic aspirin, NSAIDS. antibodies, corticosteroids, androgens or phenytoin within the past 3 months?		
Are you on anticoagulant therapy?		
Do you smoke?		
Do you consume large amounts of alcohol? (more than 2 drinks per day or 12 drinks per week) Do you follow a specific diet?		
Do you have major food allergy?		
Do you have lactose intolerance?		
Have you had major surgery in the last 6 months?		
Do you have diabetes mellitus?		
Do you have kidney disease?		
Do you have liver disease?		
Do you have heart disease?		

Do you have gastrointestinal, pancreatitis or biliary disease (onset within past three months)?		
Have you had cancer? If yes, occurrence of therapy within past 1 year?		
Do you have anemia, bleeding disorder or significant blood loss/donation?		
Do you have uncontrolled thyroid disease or hypertension? (Subject will be accepted if she is on a stable dose of a thyroid or blood pressure medication that has no known effects on blood lipid metabolism.)		
Do you have a history of eating disorders?		

E. Additional Physician Notes

Based on the medical examination and medical history, is the subject eligible to participate in the study protocol (circle one):

YES **NO**

Physician's Signature:_____

Date:_____

Appendix II

Subject Informed Consent Form

Richardson Centre for Functional Foods and Nutraceuticals

RESEARCH SUBJECT INFORMATION AND CONSENT FORM

Title of Study:

Canola Oil Multi-center Intervention Trial II (COMIT II): Effects of Oleic Acid Enriched and Regular Canola Oil on Body Composition and Lipid Metabolism in Participants with Metabolic Syndrome Risk Factors

Investigator: Peter J.H. Jones, PhD
Richardson Centre for Functional Foods and Nutraceuticals
University of Manitoba
196 Innovation Drive
Winnipeg, Manitoba R3T 6C5
Phone: 204 474 9787

Sponsor: Canola Council of Canada, 400-167 Lombard Avenue, Winnipeg, Manitoba, R3B OT6

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

Purpose of study

Obesity is a contributing factor to most chronic diseases and a burden on human health throughout the world. The ability to limit or even reduce excess weight gain through modification of fatty acid intake would be beneficial in combating the obesity epidemic. The primary objectives of the study are to examine the health benefits of dietary canola oils on body composition and cardiovascular disease risk factors. It is anticipated that consuming these healthy oils will favorably alter body composition, specifically through

the reduction of android (stomach) fat. Furthermore, we are also examining the effects of dietary canola oils on many other cardiovascular disease risk factors.

Study procedures

Pre-screening procedures

If you agree to take part in this study, you will be asked to give a fasting (nothing to eat or drink 12 hours before the test) blood sample (approximately two teaspoons) to measure your blood lipid levels and additional biochemistry parameters. In addition, we will measure your blood pressure and waist circumference. Prior to beginning the study, you will undergo a physical examination by a physician to ensure that you are in good health. During the physical examination, the physician will measure your vital signs, examine the normality of body systems and ask you some questions regarding your medical history. The study physician and or study staff will review medical history and ask questions to determine whether you are eligible to participate. Any change in your health status at any point during the study needs to be reported to the study investigators.

Study procedures

The study will consist of 3 phases of 42 days (6 weeks) each during which you will consume a fixed composition of a precisely controlled weight-maintaining diet. Each study phase will be followed by a washout period of 6 weeks where you can consume your habitual diets. During each study phase, you will be provided with a balanced, precisely controlled weight-maintaining diet (35% energy from fat, 50% carbohydrate, and 15% protein). Each phase will differ only in the dietary oils provided as part of the controlled diet as follows:

1) Control phase: Dietary fat consumed will provide 35% of total energy and will be comprised of "Western diet" fats in the form of a mixture of common dietary oils.

2) Canola oil phase: Dietary fat consumed will provide 35% of total energy and will be comprised of up to 60% of total energy from fat from *canola oil*.

3) High oleic acid canola oil phase: Dietary fat consumed will provide 35% of total energy and will be comprised of up to 60% of total energy from fat from *high oleic acid canola oil*. Oleic acid is a specific dietary fatty acid of interest with health benefits.

This study is a double-blind design, which means that neither you nor the study staff will know which oil variety you are receiving in each phase. In the unlikely event of an emergency, this information will be made available.

Study diets will be prepared in the metabolic kitchen of the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN). You will consume at least 1 of 3 daily meals at the RCFFN under supervision. The other meals will be prepared and packed to be taken out. The treatment oils will be provided as a part of the meals, as appropriate for each phase. You will be asked to consume only the food provided by our metabolic kitchen during each study phase. Additionally, we ask that you do limit caffeinated

beverages consumption to one to two beverages a day, alcoholic beverages consumption to 2 beverages a week. No alcoholic beverages are to be consumed within 48 hours prior to blood draws during the study periods. No caffeinated beverages consumption within 12 hours prior to blood draws during the study periods

We will measure the amount of fat in your body using a procedure called dual energy x-ray absorptiometry (DXA). These analyses will be performed 6 times in total during the study, once at the beginning of each phase and once at the end of each phase. For this procedure, you will need to lie in a horizontal position for about 5-10 minutes while the scan arm passes from your head to your feet. The radiation from this test is very low dosage (equivalent to approximately 1 day of natural background radiation). The dosage is 1000 times less than the limit for trivial exposure. You will be asked not to wear anything metal (metal may affect bone density values which will affect body composition calculations). In addition, you will need to ensure that you will not undergo barium tests/exams, or a nuclear medicine scan or injection with an x-ray dye within two weeks prior to your DXA scan. If you are female and are not post-menopausal you will be asked to take a pregnancy test prior to beginning the study and subsequently before each DXA scan. To measure abdominal subcutaneous and visceral fat, the DXA scan you will undergo will be used to measure this.

From the 28th to 35th day of each dietary period participants will be asked to wear activity monitors around the waist. This activity monitoring period is optional and you may choose not to participate. If you do choose to participate these small devices are about the size of a wrist watch and can be worn on a belt or with special belts that are made for the monitors. These devices measure movement and ambient light and this data will be used to measure 24 hour physical activity, energy expenditures, and sleep/wake measurements. On the 35th day of each period the data stored on the devices will be downloaded and saved under code and the data on the device will be deleted.

During days 1, 2, 41 and 42 of each six-week test diet phase, fasting blood samples will be obtained for assessment of blood fat, fatty acid profile and other cardiovascular diseases (CVD) biomarkers including insulin glucose concentrations and inflammatory markers, oxidative stress markers and markers of adiposity.

Each blood draw will require taking approximately 10 teaspoons of blood and will take approximately 5 minutes. The total amount of blood drawn during each phase of the study will be approximately 10 tablespoons. The total blood volume required for this trial will be approximately 2 cups.

On day 41, you will be required to consume a small amount of deuterated water (about 2-3 tablespoons). The movement of these tagged materials will permit assessment of the change in fatty acid metabolism of your body in response to your diet. All of the above tagged materials are non-radioactive, non-toxic, and do not pose any health risk to you.

During the last week of each phase, you will also be required to undergo a non-invasive ultrasound test called flow mediated dilatation (FMD), which will help us measure how efficiently your arteries are regulating blood flow. In addition, your arterial health will be further measured through another non-invasive procedure using a Mobil-O-Graph which will only require you to wear a blood pressure cuff which measures blood pressure at the same time as determining your blood vessel elasticity.

Risks and discomforts
As with any clinical trial, there may be as yet unknown or unforeseen risks of taking part.

The dietary oils contained within the meals at the proposed level has been shown to have no known direct negative side effects on health in several dozen existing animal and human experiments. 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.

Vascular ultrasound test discomfort and risks
There are no known risks associated with ultrasound. Measuring FMD requires the forearm blood pressure cuff to be inflated tightly. This may cause participants' hands and arms below the blood pressure cuff to go numb or feel a tingling or pricking sensation while the cuff is inflated and after it is released; it is similar to the sensation of your hand or arm "falling asleep." During the 5 minutes that the blood pressure cuff is inflated on your forearm, your arm could become numb and we will ask you not to move it. This might be moderately painful. However, any discomfort or numbness should go away within minutes of cuff deflation and there are no known long-term risks associated with this test. There is a possibility for red blotching or mild bruising (petechiae) appearing on the skin above and below the location of the blood pressure cuff. Studies in adults indicate that petechiae is rare (occurring in less than 0.5% of patients), is typically not uncomfortable, and does not require treatment. There are no risks associated with measurement of blood pressure, heart rate, or EKG as long as the participant is not allergic to adhesive tape. Paper and/or foam EKG electrodes are available for individuals with adhesive tape sensitivities. You will be given the opportunity to inspect these electrodes prior to participating so you can determine if you will have a reaction to them. Temporary redness at the site of the electrode placement is possible.

In case you feel any discomfort during the experimental trial a physician, Dr. Erica Luong, will be available to contact at any time. Dr. Erica Luong can be reached at 204 890 3441.

Benefits
You may not benefit from participation in this research; however, the study should contribute to a better understanding of the effects of dietary oils on body composition and blood fat levels as well as CVD biomarkers. You will also receive access to your test results when they become available.

Costs
All clinic and professional fees, diagnostic and laboratory tests that will be performed as part of this study are provided at no cost to you. There will be no cost for the study treatment that you will receive.

Remuneration for participation
You will receive up to a maximum of $750.00 for your time and inconvenience of the study schedule. This amount will be provided as $175.00 remunerated at the end of each of the first two phases $400.00 will be provided upon completion of the 3rd and final phase. If you withdraw early from the study, you will receive an appropriate pro-rated fraction of this amount.

Alternatives
You are not obligated 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
Medical records that contain your identity will be treated as confidential in accordance with the Personal Health Information Act of Manitoba. The RCFFN staff involved with your care may review/copy medical information that may reveal your identity. With your permission, the study doctor may also write to your Family Doctor to tell him/her that you are taking part in a study or to obtain further medical information. The Biomedical Research Ethics Board at the University of Manitoba and National Research Council Research Ethics Board may also review your research-related records for quality assurance purposes. If you are a research participant from the St. Boniface General Hospital, your research related records may be reviewed by the St. Boniface General Hospital for quality assurance purposes. Other agencies that may review your research related records for quality assurance and data analysis include; St. Boniface Research Centre, Manitoba; University of Toronto, Ontario; University of Laval, Quebec; Pennsylvania State University, Pennsylvania, United States; Canola Council of Canada; and Agriculture and Agri-Food Canada. But these agencies will not be able to link your research related data with your personal information. If the results of the trial are published, your identity will remain confidential. Personal information such as your name, address, telephone number and/or any other identifying information will not leave the RCFFN.

Study samples will be stored in a locked freezer at the RCFFN. Only the study coordinators and the principal investigator will have access to the samples. Your samples will not be used for any additional analyses, nor stored for any longer than 5 years, nor shared with any other group, other than is indicated in the protocol, without your prior specific consent.

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 to not participate or to withdraw from the study will not affect your other medical care.

Your participation in this study may be terminated without your consent by the study coordinators, physician or principal investigator. The study staff will withdraw you if he/she feels that participation is no longer in your best interest, or if you fail to follow the directions of the study staff.

If you decide to participate, you will agree to cooperate fully with the study visit schedule, and will follow the study staff's instructions.

We will tell you about new information that may affect your health, welfare, or willingness to stay in this study.

Should you wish to withdraw your participation from the study, you must inform the study coordinators so that your file can be officially close.

Medical care for injury related to 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. Erica Luong at 204 890 3441 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 nor releasing the investigator or the sponsor from their legal and professional responsibilities. If any health abnormalities are identified in the clinical tests conducted during this experiment, Dr. Erica Luong will be contacted, who will inform you of the results.

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

Investigator:	Dr. Peter Jones	Tel No.	204 474 9787
Coordinator:	Julia Rempel	Tel No.	204 480 1042
Study Physician:	Dr. Erica Luong	Tel No.	204 890 3441

For questions about your rights as a research subject, you may contact:

The Biomedical Research Ethics Board, University of Manitoba at 204 789 3389

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

This study is registered on a publicly available Registry Databank at 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.

Consent

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

Yes ☐ No ☐

1. I have read and understood this Information and Consent Form, and I freely and voluntarily agree to take part in the clinical trial (research study) described above.

2. I understand that I will be given a copy of the signed and dated Information and Consent Form. I have received an explanation of the purpose and duration of the trial, and the potential risks and benefits that I might expect. I was given sufficient time and opportunity to ask questions and to reflect back my understanding of the study to study personnel. My questions were answered to my satisfaction.

3. I agree to cooperate fully with the study doctor and will tell him if I experience any side effects, symptoms or changes in my health.

4. I am free to withdraw from the study at any time, for any reason, and without prejudice to my future medical treatment.

5. I have been assured that my name, address and telephone number will be kept confidential to the extent permitted by applicable laws and/or regulations.

6. By signing and dating this document, I am aware that none of my legal rights are being waived.

Signature:_____ Date: _____

Printed name of above:_____

I confirm that I have explained the purpose, duration etc of this clinical trial, as well as any potential risks and benefits, to the subject whose name and signature appears above. I confirm that I believe that the subject has understood and has knowingly given their consent to participate by his/her personally dated signature.

Signature:_____ Date: _____

Printed name of above:_____ Study role:_____

ALL SIGNATORIES MUST DATE THEIR OWN SIGNATURE

Appendix II

Genetic Consent Form

Richardson Centre for Functional Foods and Nutraceuticals

ADDITIONAL RESEARCH SUBJECT INFORMATION AND CONSENT FORM FOR GENETICS ANALYSIS

Title of Study: **Canola Oil Multi-center Intervention Trial II (COMIT II): Effects of Oleic Acid Enriched and Regular Canola Oil on Body Composition and Lipid Metabolism in Participants with Metabolic Syndrome Risk Factors**

Investigator: Peter Jones, PhD
Richardson Centre Functional Foods and Nutraceuticals
University of Manitoba
196 Innovation Drive, Smartpark
Winnipeg, Manitoba R3T 6C5
Phone: (204) 474-9787

Sponsor: Canola Council of Canada, 400-167 Lombard Avenue, Winnipeg, Manitoba, R3B OT6

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

The study doctor and institution are receiving professional fees and financial support to conduct this study.

Nature and duration of procedure

From the blood drawn during the clinical study as outlined in the Research Subject Information and Consent Form, we would like to extract genetic information from your cells and perform analyses using laboratory techniques that augment and recognize specific genes to determine

ADDITIONAL RESEARCH SUBJECT INFORMATION AND CONSENT FORM FOR GENETIC ANALYSIS

how your genetic makeup influences the efficiency of your body in converting dietary oil-derived fatty acids into longer chain fatty acids that are known to have health benefits, and how the intake of these dietary oil-derived fatty acids change your gene expression profile. Genetic information which includes DNA and RNA are molecules found in the cells of your body and are organized into genes that contain all of the information needed to make the proteins that perform specific biological functions in your body.

Confidentiality and safekeeping of biological samples containing genetic information

All of the information obtained about you and the results of the research will be treated confidentially. We will protect your confidentiality by assigning your samples containing genetic information a specific code. This code will link you to your samples containing genetic information and can only be decoded by the principal researcher or an individual authorized by the latter. Samples containing your genetic information will be kept at the Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba, under the supervision of Dr. Peter Jones for a 5-year period following the end of the research project. After this time, all samples will be destroyed. Your samples containing genetic information will only be used for the purpose of this research project.

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

Potential risks and/or benefits

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. Erica Luong, will be available to contact at any time. Dr. Erica Luong can be reached at 204 890 3441.

While there may be no direct benefits to you for taking part in these additional analyses, we hope that these results will provide us with the information on genetic characteristics of people in response to the intake of the treatment oils.

ADDITIONAL RESEARCH SUBJECT INFORMATION AND CONSENT FORM FOR GENETIC ANALYSIS

Signature of participant

The content of the procedure and procedures associated with it have been fully explained to me, and all experimental procedures have been identified. I have had the opportunity to ask questions concerning any and all aspects of the project and procedures involved, and may continue in the future to ask further questions at any time, as it is my right to do so. I am aware that I may refuse to participate as well as withdraw my consent at any time. I acknowledge that no guarantee or assurance has been given by anyone as to the results to be obtained and that my participation in this study is completely voluntary. Confidentiality of records concerning my involvement in this project will be maintained in an appropriate manner. Samples will not be utilized for any additional analyses, nor stored for any prolonged period, nor shared with any other group, other than is indicated in the protocol, without my specific consent.

I, _____, have read the above description. I have been made aware of all the procedures, advantages and disadvantages of the study, which have been explained to me.

_____ _____

Signature of Subject Date

I confirm that I have explained the purpose, duration etc of this clinical trial, as well as any potential risks and benefits, to the subject whose name and signature appears above. I confirm that I believe that the subject has understood and has knowingly given their consent to participate by his/her personally dated signature.

Signature:_____ Date/Time: _____

Printed name of above:_____ Study role:_____

Appendix II

Subject Information Sheet

COMIT II Trial Instructions

Controlled Feeding Helpful Hints

We recognize that participating in a controlled feeding study is a significant commitment so we have put together some helpful hints to guide you along the way. But please, if you have any problems, concerns or questions please ask any of the staff – we will try to help you as best we can.

Study Contact Info: please let us know as soon as possible if you are not coming at your "regular" time, are having any problems or need to fill out a "pack out request" (to have meals packed out for a trip or meeting).

Allowed Beverages

1) Caffeine-free diet or unsweetened beverages may be consumed in any amount desired. These include:
   Water
   Calorie-free mineral water
   Diet caffeine-free soda
   Crystal Light or sugar-free KoolAid (the Crystal Light cannot be the fortified kind)
   Decaf Coffee and Tea

2) Caffeinated no-calorie diet soda beverages and caffeinated coffee and tea are limited to:
   2 servings per day
   Diet Soda - 1 serving is one 12 oz can
   Coffee and tea - 1 serving is 8 oz

Beverages Not Allowed

1) Alcoholic beverages are limited to 2/week for this study. (1 alcoholic drink is considered to be a 12 oz beer, 5 oz of wine, or 1.5 oz of hard liquor and if you drink hard liquor the mixer must be non-caloric, i.e., diet soda, water, etc.)

2) Regular soda or beverages with calories are not allowed, this includes all regular sodas, fruit juices, vegetable juices, milk etc.

Allowed Seasonings & Sweeteners

1) You may use the following seasonings as desired:

Lemon Pepper	Pepper	**Salt-free** seasonings
Mrs. Dash	Lemon Juice	Tabasco or hot pepper sauce

2) The following condiments are allowed in limited amounts (you may have up to 5 units/day). One unit is listed for each.
   - Ketchup - 1 packet
   - Mustard - 2 packets
   - Horseradish - 1 tbsp

3) Sweeteners:
   - Any non-caloric sweetener, e.g., Sweet-n-Low, Equal

Sweeteners Not Allowed

The following sweeteners are **not** allowed:
- Any sweetener with calories , e.g., sugar of any kind (brown, raw, white etc), honey

Allowed Medications

During the study, you will be asked on a daily basis if you have been ill and if so, have you taken any medication. If necessary, and on an occasional basis, it is OK to take over-the-counter and prescription medication as listed below. For any medication not listed below, please ask.

Headache/Pain Medications	Tylenol – check before taking any other pain medication (such as Advil, Ibuprofen, etc)
Sleep/Sedative Medications	OTC Preparations – check with study staff
Cold/Allergy Medications	Check with study staff
Laxatives	Senna - only for occasional use
Antidiarrheal	Lomotil, Kaopectate - only for occasional use.
Cough	Check with study staff

Do not take Aspirin, or vitamin/herb supplements. If you need to take an antibiotic, please check with the study staff before taking it.

Gum

Sugar-free chewing gum is allowed.

Subject Information Sheet

Fasting Blood Draws

Please be sure to fast for 12 hrs prior to each blood draw. You may only drink water during these fasting periods (we advise you to drink plenty of water 2 days prior to your visit as this will facilitate blood sampling). **Also, please do not drink alcohol for 48 hrs prior to each blood draw, and caffeinated beverages 12 hrs prior to each blood draw**. We will remind you when the blood draws are approaching so that you will remember about the fasting and alcohol restriction.

Exercise

Please do not alter your level of physical activity during the study. Ideally, we would like you to maintain a consistent level of activity, with very few changes to your normal routine. It also is important not to engage in very strenuous activity (i.e. aerobics class, jogging, etc) on the day before a blood draw.

HINTS FOR THE STUDY:

1) If you are a coffee or tea drinker, you may use some of the milk from your breakfast in your beverage. You may NOT use additional milk.
2) You may save some breakfast butter for use in that day's dinner. There is the entrée, and usually a vegetable and dinner roll to divide it up between if you feel there is too much for breakfast.

Thanks for your participation!!!! Questions??

Please ask study staff:
Phone: 204-480-1042 (available Monday-Friday 8:30-4:30)
Email: canolatrial2@gmail.com

Appendix II

Study Weight Monitoring Form

COMIT II Study Weight Monitoring

Subject Code:
Phase:

Date	Weight (Lbs)
6-Nov-14	
7-Nov-14	
10-Nov-14	
11-Nov-14	
12-Nov-14	
13-Nov-14	
14-Nov-14	
17-Nov-14	
18-Nov-14	
19-Nov-14	
20-Dec-14	
…….	

Appendix II

Study Phase Schedule

COMIT II Study Schedule

Sun	Mon	Tue	Wed	Thurs	Fri	Sat
17	18	19	*August 20* •12 h fast	21 START DATE •Blood draw •Record your weight •B/fast at RCFFN •12 h fast •DXA scan	22 •DXA scan •Blood draw •Record your weight •B/fast at RCFFN	*23* •Food delivered
24 •Food delivered	25 •Record your weight •Breakfast at RCFFN	26 •Record your weight •Breakfast at RCFFN	27 •Record your weight •Breakfast at RCFFN	28 •Record your weight •Breakfast at RCFFN	*29* •Record your weight •Breakfast at RCFFN •Weekly monitoring form	*30* •Food delivered
31 •Food delivered	*September 1* •Record your weight •Breakfast at RCFFN •Food delivered	2 •Record your weight •Breakfast at RCFFN	3 •Record your weight •Breakfast at RCFFN	4 •Breakfast at RCFFN	5 •Breakfast at RCFFN •Weekly monitoring form	*6* •Food delivered
7 •Food delivered	8 •Breakfast at RCFFN	9 •Breakfast at RCFFN	10 •Breakfast at RCFFN	11 •Breakfast at RCFFN	12 •Record your weight •Breakfast at RCFFN •Weekly monitoring form	*13* •Food delivered
14 •Food delivered	15 •Breakfast at RCFFN	16 •Breakfast at RCFFN	17 •Breakfast at RCFFN •Optional activity monitor given	18 •Breakfast at RCFFN •Activity monitor	19 •Record your weight •Breakfast at RCFFN •Weekly monitoring form •Activity monitor	*20* •Food delivered •Activity monitor
21 • Food Delivered • Activity monitor	22 •Breakfast at RCFFN •Activity monitor	23 •Breakfast at RCFFN •Activity monitor	24 •Breakfast at RCFFN •Activity monitor	25 •Breakfast at RCFFN •Return activity monitor	26 •Record your weigh: •Breakfast at RCFFN •Weekly monitoring form	*27* •Food delivered
28 •Food delivered	29 •Breakfast at RCFFN •12 h fast	30 •Record your weight •Breakfast at RCFFN •DXA •Blood draw •Heavy water •Record your weight •Breakfast at RCFFN •12 h fast	*October 1* END DATE •DXA •Blood draw •Record your weight •Breakfast at RCFFN		•Mobil-o-Graph to be completed in week 5 of study (optional)	

No caffeine 12 h prior to blood draw; No alcohol 48 hours prior to blood draw

No caffeine 12 h prior to blood draw; No alcohol 48 hours prior to blood draw

Appendix II

Shake Consumption Checklist

Participant Shake Checklist

Phase 1

Participant Code:

Date	Shake taken?		Comments
	Participant Initial	Coordinator Initial	
06-Feb-14			
07-Feb-14			
10-Feb-14			
11-Feb-14			
12-Feb-14			
13-Feb-14			
14-Feb-14			
17-Feb-14			
18-Feb-14			
19-Feb-14			
20-Feb-14			
21-Feb-14			
24-Feb-14			
25-Feb-14			
26-Feb-14			
27-Feb-14			
28-Feb-14			
03-Mar-14			
……			

Appendix II

Data Collection and Monitoring Forms

COMIT II DATA COLLECTION AND MONITORING FORMS

Participant Code:_____ Phase: ____

Participant Code _____

Study Phase _____ Treatment (i.e., A, B, C) _____

Start Date _____ End Date _____

SECTION 1: START AND END WEIGHT

Instructions: *Ask participants to remove heavy footwear and/or heavy articles of clothing (i.e., jackets, outerwear), before each weight measurement.*

Day 1: Weight (lbs) _____ Staff Initials _____

Day 2: Weight (lbs) _____ Staff Initials _____

Day 41: Weight (lbs) _____ Staff Initials _____

Day 42: Weight (lbs) _____ Staff Initials _____

SECTION 2: WAIST CIRCUMFERENCE

Instructions: *Waist circumference is measured at either the natural waist, i.e., smallest circumference of the abdomen, or midway between the lowest rib and iliac crest. The measuring tape should be snug around the body, but not pulled so tight that it is constricting.*

Day 1 or 2:

a. First measurement (cm): _____

b. Second measurement (cm): _____

c. Average (1st and 2nd) waist circumference measurement: _____

Staff Initials _____

Day 41 or 42:

a. First measurement (cm): _____

b. Second measurement (cm): _____

c. Average (1st and 2nd) waist circumference measurement: _____

Staff Initials _____

SECTION 3: SEATED BLOOD PRESSURE

Has the participant consumed caffeine, used any medication or eaten any food in the last 12 hours, and/or exercised in the past 2 hours? □ Yes* □ No

*If yes, participant needs to be rescheduled for a blood pressure measurement.

Instructions: *The participant should be instructed to relax as much as possible; ideally, at least 5 minutes should elapse before the first reading is taken. Apply cuff to non-dominant arm. After applying the cuff, the participant must be quiet and remain continuously seated without legs crossed for 5 minutes. Instruct the participant not to talk during the reading. Wait 3 minutes after each reading before taking the next reading.*

Day 1 or 2:

a. First blood pressure measurement (mmHg): _ _ _ / _ _ _ (SBP/DBP)
b. Second blood pressure measurement (mmHg): _ _ _ / _ _ _ (SBP/DBP)
c. Third blood pressure measurement (mmHg): _ _ _ / _ _ _ (SBP/DBP)
d. Average (2nd and 3rd) blood pressure measurement: _ _ _ / _ _ _ (SBP/DBP)

Staff Initials _____

Day 41 or 42:

a. First blood pressure measurement (mmHg): _ _ _ / _ _ _ (SBP/DBP)
b. Second blood pressure measurement (mmHg): _ _ _ / _ _ _ (SBP/DBP)
c. Third blood pressure measurement (mmHg): _ _ _ / _ _ _ (SBP/DBP)
d. Average (2nd and 3rd) blood pressure measurement: _ _ _ / _ _ _ (SBP/DBP)

Staff Initials _____

SECTION 4: BLOOD COLLECTION

DAY 1

Date (Month Day, Year) _____ Time (HH:MM) _____

Fasted over last 12 hours? Yes ___ / No ___

Caffeine in last 12 hours? Yes ____ / No ___ If Yes, when _____

Alcohol in last 48 hours? Yes ____ / No ____ If Yes, when _____

Arm Right ____ / Left____

Collect:
2 X 7.5 ml serum (red/grey SST tube) _____ (day 1, 2, 41, 42)
2 X 4 ml plasma heparin (green top) _____ (day 1, 2, 41, 42)
2 X 4 ml plasma EDTA (purple top) _____ (day 1, 2, 41, 42)

Have all blood samples been obtained? Yes ___ / No** ____

*** If No, please state reasons why:* _____

_____

_____

_____

Ease of Blood Draw: Excellent ____ Good ____ Difficult _____ Very Difficult _____

Comments (Anything which could impact blood draw results? i.e., Are you feeling stressed? How did you sleep last night? Has anything out of the ordinary happened over the past 48 hours?)**:**_____

_____

_____

_____

Name of Phlebotomist:_____

SECTION 4: BLOOD COLLECTION (continued)

DAY 2

Date (Month Day, Year) _____ Time (HH:MM) _____

Fasted over last 12 hours? Yes ___ / No ___

Caffeine in last 12 hours? Yes ____ / No ___ If Yes, when _____

Alcohol in last 48 hours? Yes ____ / No ____ If Yes, when _____

Arm Right ____ / Left____

Collect:
2 X 7.5 ml serum (red/grey SST tube) _____ (day 1, 2, 41, 42)
2 X 4 ml plasma heparin (green top) _____ (day 1, 2, 41, 42)
2 X 4 ml plasma EDTA (purple top) _____ (day 1, 2, 41, 42)

Have all blood samples been obtained? Yes ___ / No** ____

*** If No, please state reasons why:* _____

_____

_____

_____

Ease of Blood Draw: Excellent ____ Good ____ Difficult _____ Very Difficult _____

Comments (Anything which could impact blood draw results? i.e., Are you feeling stressed? How did you sleep last night? Has anything out of the ordinary happened over the past 48 hours?)**:**_____

_____

_____

_____

Name of Phlebotomist:_____

SECTION 4: BLOOD COLLECTION (continued)

DAY 41

Date (Month Day, Year) _____ Time (HH:MM) _____

Fasted over last 12 hours? Yes ___ / No ___

Caffeine in last 12 hours? Yes ____ / No ___ If Yes, when _____

Alcohol in last 48 hours? Yes ____ / No ____ If Yes, when _____

Deuterium water provided?* Yes ____ / No ____ ***Only required on day 41 of each phase***

Arm Right ____ / Left____

Collect:
2 X 7.5 ml serum (red/grey SST tube) _____ (day 1, 2, 41, 42)
2 X 4 ml plasma heparin (green top) _____ (day 1, 2, 41, 42)
2 X 4 ml plasma EDTA (purple top) _____ (day 1, 2, 41, 42)

Have all blood samples been obtained? Yes ___ / No** ____

*** If No, please state reasons why:* _____

_____

_____

_____

Ease of Blood Draw: Excellent ____ Good ____ Difficult _____ Very Difficult _____

Comments (Anything which could impact blood draw results? i.e., Are you feeling stressed? How did you sleep last night? Has anything out of the ordinary happened over the past 48 hours?)**:**_____

_____

_____

_____

Name of Phlebotomist:_____

SECTION 4: BLOOD COLLECTION (continued)

DAY 42

Date (Month Day, Year) _____ Time (HH:MM) _____

Fasted over last 12 hours? Yes ___ / No ___

Caffeine in last 12 hours? Yes ____ / No ___ If Yes, when _____

Alcohol in last 48 hours? Yes ____ / No ____ If Yes, when _____

Arm Right **___** / Left**___**

Collect:
2 X 7.5 ml serum (red/grey SST tube) _____ (day 1, 2, 41, 42)
2 X 4 ml plasma heparin (green top) _____ (day 1, 2, 41, 42)
2 X 4 ml plasma EDTA (purple top) _____ (day 1, 2, 41, 42)
1 X 2.5 ml whole blood (PAXgene tube) ____ (day 42)

Have all blood samples been obtained? Yes ___ / No** ____

*** If No, please state reasons why:*

☐ No PAXgene tube collected since genetic analysis consent not given

☐ Other (please describe): _____

_____

_____

_____

Ease of Blood Draw: Excellent ____ Good ____ Difficult _____ Very Difficult _____

Comments (Anything which could impact blood draw results? i.e., Are you feeling stressed? How did you sleep last night? Has anything out of the ordinary happened over the past 48 hours?)

:_____

_____

_____

_____

Name of Phlebotomist:_____

SECTION 5: DXA

To be completed by study staff:

BASELINE DXA, DAY: 1 2 3 (please circle one)

Date (Month Day, Year) _____ Time of scan (HH:MM): _____

Since screening has the volunteer had any radiation exposure (e.g. x-rays, radiotherapy, CT scan)? Yes ___ / No ___

Date & details: _____

_____

_____

FEMALES OF CHILDBEARING AGE ONLY:

Possibility of being pregnant Yes ___ / No ___

Has a pregnancy test been offered? Yes ___ / No ___

Has a test been taken? Yes ___ / No*** ___ Test Results (circle): **Negative / Positive**

Result Confirmation:

Participant signature:_____ Study staff signature:_____

***If no pregnancy test taken please explain why:

_____

_____

Check:

Jewelry removed (including glasses): Yes ___ / No ___

Spine straight: Yes ___ / No ___

Comments:_____

_____

_____

Staff Initials:_____

SECTION 5: DXA (continued)

To be completed by study staff:

ENDPOINT DXA, DAY: 40 41 42 (please circle one)

Date (Month Day, Year): _____ Time of scan (HH:MM): _____

Since screening has the volunteer had any radiation exposure (e.g. x-rays, radiotherapy, CT scan)? Yes ___ / No ___

Date & details: _____

_____

_____

FEMALES OF CHILDBEARING AGE ONLY:

Possibility of being pregnant Yes ___ / No ___

Has a pregnancy test been offered? Yes ___ / No ___

Has a test been taken? Yes ___ / No*** ___ Test Results (circle): **Negative / Positive**

Result Confirmation:

Participant signature:_____ Study staff signature:_____

***If no pregnancy test taken please explain why:

_____

_____

Check:

Jewelry removed (including glasses): Yes ___ / No ___

Spine straight: Yes ___ / No ___

Comments:_____

_____

_____

Staff Initials:_____

SECTION 6: ACTIVITY MONITORS

☐ Consent given to participate in activity monitoring

☐ **No** consent given to participate in activity monitoring

Contact Dylan Mackay (Dylan.Mackay@umanitoba.ca) for assistance in set-up and programming of the activity monitors.

Instructions

- *The ActigGraph GT3X+ activity monitors need to be charged using the USB cables until the green LED light has stopped blinking.*
- *The monitors then need to be programed using the ActiLife 6 software. Each monitor will be programed for a 7 day period of measurement, starting at 12:00 am on day 29 of each dietary period and running until 12:00am on day 36. With a 30 hz epoch sample rate and triaxial (3 axis enabled) monitoring.*
- *The monitors will be programmed with the participant code, height, weight, gender, race and date of birth. The serial number of the monitor used for each participant will be recorded in an Excel sheet so that the same monitor can be used by each participant throughout the study.*
- *The monitors will be given to the participants on day 28 of each study period and collected from the participants on or after day 36.*
- *The "Instructions for Activity Monitors" sheet should be given and explained to participants each time they are starting to wear the activity monitors.*
- *After collection the monitor data will be offloaded and saved in the ActiLife data vault, the data files will be named by participant code and the date of day 36 of the dietary period for which the activity data was collected. ie. C212 (2014-02-07).gtx (year-month-day).*

SECTION 7: WEEKLY MONITORING FORMS

WEEK 1

1. In the past week has your exercise level changed? ☐ Yes ☐No

If Yes, was it: ☐ More Active ☐ Less Active ☐ No Exercise

***Please remember to keep your exercise level constant throughout the study.**

2. Have you taken any prescription or non-prescription drugs in the past week? ☐ Yes ☐No

If Yes, specify:

Name	Dosage	Frequency	Reason	Start Date	End Date

3. Have you taken any vitamins, minerals or other supplements in the past week? ☐ Yes ☐No

If Yes, specify:

Name	Dosage	Frequency	Reason	Start Date	End Date

4. Have you had any changes in a medical condition, new illness or injury in the past week? ☐ Yes ☐No

If Yes, describe: _____

5. If you were ill in the past week, did your eating change as a result? ☐ Yes ☐No

If Yes, describe: _____

6. Have you eaten any foods outside of the study diet? ☐ Yes ☐No

If Yes, describe: _____

7. Any specific comments regarding study food: _____

_____

_____

_____

SECTION 7: WEEKLY MONITORING FORMS (continued)

WEEK 2

1. In the past week has your exercise level changed? ☐ Yes ☐No

If Yes, was it: ☐ More Active ☐ Less Active ☐ No Exercise

***Please remember to keep your exercise level constant throughout the study.**

2. Have you taken any prescription or non-prescription drugs in the past week? ☐ Yes ☐No

If Yes, specify:

Name	Dosage	Frequency	Reason	Start Date	End Date

3. Have you taken any vitamins, minerals or other supplements in the past week? ☐ Yes ☐No

If Yes, specify:

Name	Dosage	Frequency	Reason	Start Date	End Date

4. Have you had any changes in a medical condition, new illness or injury in the past week? ☐ Yes ☐No

If Yes, describe: _____

5. If you were ill in the past week, did your eating change as a result? ☐ Yes ☐No

If Yes, describe: _____

6. Have you eaten any foods outside of the study diet? ☐ Yes ☐No

If Yes, describe: _____

7. Any specific comments regarding study food: _____

_____

_____

_____

SECTION 7: WEEKLY MONITORING FORMS (continued)

WEEK 3

1. In the past week has your exercise level changed? ☐ Yes ☐No

If Yes, was it: ☐ More Active ☐ Less Active ☐ No Exercise

***Please remember to keep your exercise level constant throughout the study.**

2. Have you taken any prescription or non-prescription drugs in the past week? ☐ Yes ☐No

If Yes, specify:

Name	Dosage	Frequency	Reason	Start Date	End Date

3. Have you taken any vitamins, minerals or other supplements in the past week? ☐ Yes ☐No

If Yes, specify:

Name	Dosage	Frequency	Reason	Start Date	End Date

4. Have you had any changes in a medical condition, new illness or injury in the past week? ☐ Yes ☐No

If Yes, describe: _____

5. If you were ill in the past week, did your eating change as a result? ☐ Yes ☐No

If Yes, describe: _____

6. Have you eaten any foods outside of the study diet? ☐ Yes ☐No

If Yes, describe: _____

7. Any specific comments regarding study food: _____

_____

_____

_____

SECTION 7: WEEKLY MONITORING FORMS (continued)

WEEK 4

1. In the past week has your exercise level changed? ☐ Yes ☐No

If Yes, was it: ☐ More Active ☐ Less Active ☐ No Exercise

***Please remember to keep your exercise level constant throughout the study.**

2. Have you taken any prescription or non-prescription drugs in the past week? ☐ Yes ☐No

If Yes, specify:

Name	Dosage	Frequency	Reason	Start Date	End Date

3. Have you taken any vitamins, minerals or other supplements in the past week? ☐ Yes ☐No

If Yes, specify:

Name	Dosage	Frequency	Reason	Start Date	End Date

4. Have you had any changes in a medical condition, new illness or injury in the past week? ☐ Yes ☐No

If Yes, describe: _____

5. If you were ill in the past week, did your eating change as a result? ☐ Yes ☐No

If Yes, describe: _____

6. Have you eaten any foods outside of the study diet? ☐ Yes ☐No

If Yes, describe: _____

7. Any specific comments regarding study food: _____

_____

_____

_____

SECTION 7: WEEKLY MONITORING FORMS (continued)

WEEK 5

1. In the past week has your exercise level changed? ☐ Yes ☐No

If Yes, was it: ☐ More Active ☐ Less Active ☐ No Exercise

***Please remember to keep your exercise level constant throughout the study.**

2. Have you taken any prescription or non-prescription drugs in the past week? ☐ Yes ☐No

If Yes, specify:

Name	Dosage	Frequency	Reason	Start Date	End Date

3. Have you taken any vitamins, minerals or other supplements in the past week? ☐ Yes ☐No

If Yes, specify:

Name	Dosage	Frequency	Reason	Start Date	End Date

4. Have you had any changes in a medical condition, new illness or injury in the past week? ☐ Yes ☐No

If Yes, describe: _____

5. If you were ill in the past week, did your eating change as a result? ☐ Yes ☐No

If Yes, describe: _____

6. Have you eaten any foods outside of the study diet? ☐ Yes ☐No

If Yes, describe: _____

7. Any specific comments regarding study food: _____

_____

_____

_____

SECTION 7: WEEKLY MONITORING FORMS (continued)

WEEK 6

1. In the past week has your exercise level changed? ☐ Yes ☐No

If Yes, was it: ☐ More Active ☐ Less Active ☐ No Exercise

***Please remember to keep your exercise level constant throughout the study.**

2. Have you taken any prescription or non-prescription drugs in the past week? ☐ Yes ☐No

If Yes, specify:

Name	Dosage	Frequency	Reason	Start Date	End Date

3. Have you taken any vitamins, minerals or other supplements in the past week? ☐ Yes ☐No

If Yes, specify:

Name	Dosage	Frequency	Reason	Start Date	End Date

4. Have you had any changes in a medical condition, new illness or injury in the past week? ☐ Yes ☐No

If Yes, describe: _____

5. If you were ill in the past week, did your eating change as a result? ☐ Yes ☐No

If Yes, describe: _____

6. Have you eaten any foods outside of the study diet? ☐ Yes ☐No

If Yes, describe: _____

7. Any specific comments regarding study food: _____

_____

_____

_____

ADVERSE EVENT REPORTS

Description	Start Date	Intensity*	Outcome	Date Ended	Diet Related?

**Mild: An event that is easily tolerated by the volunteer, causing minimal discomfort and not interfering with everyday activities*
Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities
Severe: An event which is incapacitating and prevents normal everyday activities

STUDY COMMENTS & PROTOCOL DEVIATIONS

Date **(Month Day, Year)**	**Comments**	**Study Personnel Initials**

Appendix II:

Participant Status Summary

STATUS SUMMARY

☐ Participant completed the study

☐ Participant withdrew from the study Date of withdrawal: _____

REASON FOR WITHDRAWAL. Tick appropriate box(es):

☐ Informed consent withdrawn by the participant
☐ Failure to comply with study requirements
☐ Investigator decision to withdraw participant

Provide rationale for withdrawal (if applicable):

_____

_____

_____

_____

☐ Participant's final results sent

☐ Statement supplier form completed

Investigator Initials _____

Appendix II

Instructions for blood sample processing used for samples handling corresponding to Chapters III, IV, V, VI

COMIT II blood sample processing and collection instructions

Sample	Blood collection tube	Tube volume	Processing instructions	Aliquoting instructions	Study days
Serum	Red/grey SST tube	2 x 7.5 mL	1. Invert 5 times 2. Room temp for 30 min 3. Spin for 10 min @ 1000 x *g*	1. Aliquot serum into cryovials[1] with **brown**[2] caps (0.5mL/tube) 2. Store at -80ºC	1,2,41,42
Plasma heparin	Green top (lithium heparin)	2 x 4 mL	1. Invert 8 times 2. Spin immediately for 10 min @1300 x *g*	1. Aliquot plasma into cryovials with **green**[3] caps (0.5mL/tube) 2. Aliquot WBC (buffy coat) into 1 (one) Cryo.s™(RNase and DNase free vials)[4] 3. Aliquot RBC into cryovials with **red**[5] caps (0.5mL/tube) 4. Store all fractions at -80ºC	1,2,41,42
Plasma EDTA	Purple top (K2 EDTA)	2 x 4 mL	1. Invert 8 times 2. Spin immediately for 10 min @ 1300 x g	1. Aliquot plasma in cryovials with **purple**[6] caps (0.5ml/tube) 2. Aliquot WBC (buffy coat) in 1 (one) Cryo.s™(RNase and DNase free vials)[4] 3. Aliquot RBC into cryovials with **red**[5] caps (0.5mL/tube) 4. Store all fractions at -80ºC	1,2,41,42
Whole blood	PAXgene tube[7]	1 x 2.5 mL	1. Invert 8 times 2. Store tube upright at room temp for 2 hours	1. Store at -20 ºC for 24hr, then transfer to -80ºC	42

[1] Cryovials: Microtubes, 0.5ml with skirted base, without screw cap, bag of 500, Sarstedt #72.730.007
[2] Brown cap: Screw cap for microtubes, color coded, brown, bag of 500, Sarstedt #65.716.009
[3] Green cap: Screw cap for microtubes, color coded, green, bag of 500, Sarstedt #65.716.005
[4] RNase and DNase free vials: Cryo.s™ Cryogenic Storage Vials, Polypropylene, Sterile, Greiner Bio-One, 2ml, case of 500, VWR #82050-206.
[5] Red cap: Screw cap for microtubes, color coded, red, bag of 500, Sarstedt #65.716.003
[6] Violet cap: Screw cap for microtubes, color coded, violet, bag of 500, Sarstedt #65.716.008
[7] PAXgene Blood RNA tube, 2.5 mL, Qiagen Product #762165

Appendix III

Information relation to treatments and diet provided in Chapters III, IV, V, VI

Appendix III: Data sheet of high-oleic canola oil

RICHARDSON

TECHNICAL DATA SHEET

CANOLA HARVEST HIGH OLEIC LOW LINOLENIC CANOLA OIL

PRODUCT DESCRIPTION / DESCRIPTION DE PRODUIT : High Oleic Low Linolenic canola oil

SPECIFICATIONS / CARACTÉRISTIQUES :	
Free Fatty Acid, % Oleic / Acide gras libre, % oléique	0.05 Maximum
Color, 5 ¼ " Lovibond/ Couleur, 5 ¼ " Lovibond	1.5 Red/15 Yellow Maximum
Peroxide Value, Meq./kg, (when packed)/ Teneur en peroxyde, meq. /kg, (une fois emballés)	0.5 Maximum
Smoke Point/ Point de formation de fumée	232 °C Minimum
Iodine Value, Wijs Method/ Teneur en iode, méthode de Wijs	95-102
Moisture, Karl Fischer/ Humidité, analyse Karl Fischer	0.05% Maximum
Flavor/ Saveur	Good
Clarity/ Clarté	Clean and Free from foreign and Insoluble Material
OSI	37.5 hrs Minimum
Cold Test/ L'essai au froid	Clear after 12 hrs
Storage & Handling/ Stockage et manipulation	18.9 ° C - 23.9 °C (66 °F -75°F) – Room Temperature
Shelf Life/ Durée de conservation	12 months
Kosher Certification/ Certification cachère	Kosher Certified

MICROBIOLOGY/MICROBIOLOGIE (cfu/g): Oil has an extremely low water activity that results in the product not being used as a nutrient source for microbes. In order for a microorganism to live and grow, an aqueous phase must be present. The moisture content of oil is below 0.1%. Food products at moisture levels below 0.1%, such as canola oil, will not support any microbial growth including *E.coli*, Salmonella or yeasts & molds

INGREDIENTS :	**INGRÉDIENTS :**
High oleic low linolenic canola oil	Huile de canola à haute teneure oléique at à faible teneure linoléique

CODE DATING
Indicates date of production of the product.

The first number signifies the quarter of the year (1-4); the second number signifies the month within the quarter (1-3); the third number and fourth numbers signify the day of the month (1-31); and the last number signifies the year.

Example:
Lot **21068** represents April 06,2008

FOOD SAFETY&QUALITY SYSTEM/ SYSTEMES DE SECURITE ALIMENTAIRE ET DE QUALITE:

Richardson Oilseed Limited is fully compliant with the HACCP program requirements of the Food Safety Enhancement Program of the Canadian Food Inspection Agency. This product is manufactured and monitored under safe, sanitary conditions to prevent contamination in compliance with Food Safety and Quality Systems and Good Manufacturing Practices.
Richardson Oilseed Limited a reçu entière reconnaissance de la part de l'Agence Canadienne d'inspection des aliments (ACIA) pour son programme HACCP. Ce produit est fabriqué conformément aux bonnes pratiques de fabrication et manufacturé et surveillé dans des conditions sûres et sanitaires pour empêcher la contamination conformément aux systèmes de sécurité alimentaire et de qualité.

Appendix III: Data sheet of high-oleic canola oil

RICHARDSON

TECHNICAL DATA SHEET

CANOLA HARVEST HIGH OLEIC LOW LINOLENIC CANOLA OIL

NUTRITION FACTS / VALEUR NURITIVE	Per 100 mL	Per 10 mL (2 tsp) / Per 14 mL Par 2 c. à thé (10 mL)	
	Amount/ Teneur	Amount / Teneur	% Daily Value / Valeur Quotidienne
Calories / Calories	830	80	
Total Fat / Lipides	92 g	9 g	14%
Saturates / saturés	6 g	0.5 g	3%
+ Trans / trans	0.7g	0 g	
Polyunsaturates / polyinsaturés	14 g	1.5 g	
Omega-6 / oméga-6	12g	1.0 g	
Omega-3 / oméga-3	1.5 g	0.2 g	
Monounsaturates / monoinsaturés	66 g	7 g	
Cholesterol / Cholestérol	0 mg	0 mg	
Sodium / Sodium	0 mg	0 mg	
Potassium / Potassium	0 mg	0 mg	
Carbohydrate / Glucides	0 g	0 g	0 %
Fibre / Fibres	0 g	0 g	
Sugars / Sucres	0 g	0 g	
Protein / Protéines	0 g	0 g	
Vitamin A / Vitamine A			
Vitamin C / Vitamine C			
Calcium / Calcium			
Iron / Fer			
Vitamin D / Vitamine D			
Vitamin E / Vitamine E			15 %

NOTE:
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PRODUCT SPECIFICATIONS / CARACTÉRISTIQUES DE PRODUIT :					
Size	930 kg Tote	190 Kg Drum			
Richardson Product Number	523 1 69 02	523 1 57 04			
UPC	-	59842-40574			
SCC	099482403119	099482403119			
Unit Size	1020 kg	930 kg			
Unit Dimensions LxWxH *(cm)*	113.5 x 121.92	111 x 103			
(inches)	*44.7 x 48.0*	43.7 x 40.6			
Units / Case	N/A	N/A			
Case Weight	1020 kg	984.90 Kg			
Case Cube	1707599 cm3	1280496 cm3			
Case Dimensions LxWxH *(cm)*	123.4 x 113.5 x 121.92	112 x 111 x 103			
(inches)	*48.6 x 44.7 x 48.0*	44.1 x 43.7 x 40.6			
Units / Pallet	1 X 1	1 X 1			
Pallet Pattern	1 X 1	1 X 1			
Pallet Dimensions LxWxH *(cm)*					
(inches)					
Pallet Cube					
Pallet Weight					

For further information, please contact your Richardson Oilseed representative

Phone : 1-800-635-3296 **www.canola.com** **www.richardson.ca**

Appendix III: Data sheet of regular canola oil

RICHARDSON

TECHNICAL DATA SHEET

CANOLA HARVEST 100% CANOLA OIL

PRODUCT DESCRIPTION / DESCRIPTION DE PRODUIT :	Canola oil

SPECIFICATIONS / CARACTÉRISTIQUES :	
Free Fatty Acid, % Oleic / Acide gras libre, % oléique	0.05 Maximum
Color, 5 ¼ " Lovibond/ Couleur, 5 ¼ " Lovibond	1.5 Red/15 Yellow Maximum
Peroxide Value, Meq./kg, (when packed)/ Teneur en peroxyde, meq. /kg, (une fois emballés)	0.5 Maximum
Smoke Point/ Point de formation de fumée	232 ◦C Minimum
Iodine Value, Wijs Method/ Teneur en iode, méthode de Wijs	108 - 126
Moisture, Karl Fischer/ Humidité, analyse Karl Fischer	0.05% Maximum
Flavor/ Saveur	Good
Clarity/ Clarté	Clean and Free from foreign and Insoluble Material
OSI	8.33 hrs Minimum
Cold Test/ L'essai au froid	Clear after 12 hrs
Storage & Handling/ Stockage et manipulation	18.9 ° C - 23.9 °C (66 °F -75°F) – Room Temperature
Shelf Life/ Durée de conservation	12 months
Kosher Certification/ Certification cachère	Kosher Pareve Certified

MICROBIOLOGY/MICROBIOLOGIE (cfu/g): Oil has an extremely low water activity that results in the product not being used as a nutrient source for microbes. In order for a microorganism to live and grow, an aqueous phase must be present. The moisture content of oil is below 0.1%. Food products at moisture levels below 0.1%, such as canola oil, will not support any microbial growth including *E.coli*, Salmonella or yeasts & molds

INGREDIENTS :

Canola Oil

INGRÉDIENTS :

Huile de Canola

CODE DATING (1)

Applies to the following Richardson Product Numbers :

523 1 47 30 / 523 1 64 30 / 523 1 69 10 / 523 1 69 01 / 523 1 45 30

Indicates date of production of the product.

The first number signifies the quarter of the year (1-4); the second number signifies the month within the quarter (1-3); the third number and fourth numbers signify the day of the month (1-31); and the last number signifies the year.

Example:
Lot **21068** represents April 06,2008

CODE DATING (2)

Applies to the following Richardson Product Numbers :

521 1 44 10 / 521 1 49 10 / 521 1 09 10 / 521 1 07 10

Code on BOTTLE- BEST BY 2013 MR 18 12:00
(1 Year Expiry – BEST BY YYYY MM DD, Time)

Code on CASE- CANOLA HARVEST xxxxx
(Brand, UPC)
0001 BEST BY 2013 MR 18 12:00
(1 Year Expiry - Case ct, YYYY MM DD, time)

FOOD SAFETY&QUALITY SYSTEM/ SYSTEMES DE SECURITE ALIMENTAIRE ET DE QUALITE:

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Appendix III: Data sheet of regular canola oil

RICHARDSON

TECHNICAL DATA SHEET

CANOLA HARVEST 100% CANOLA OIL

NUTRITION FACTS / VALEUR NURITIVE	Per 100 mL	Per 10 mL (2 tsp) / Par 2 c. à thé (10 mL)	
	Amount/ Teneur	Amount / Teneur	% Daily Value / Valeur Quotidienne
Calories / Calories	830	80	
Total Fat / Lipides	92 g	9 g	14%
Saturates / saturés	7 g	0.5 g	3%
+ Trans / trans	1 g	0 g	
Polyunsaturates / polyinsaturés	26 g	2.5 g	
Omega-6 / oméga-6	17g	1.5 g	
Omega-3 / oméga-3	8 g	0.7 g	
Monounsaturates / monoinsaturés	55 g	6 g	
Cholesterol / Cholestérol	0 mg	0 mg	
Sodium / Sodium	0 mg	0 mg	
Potassium / Potassium	0 mg	0 mg	
Carbohydrate / Glucides	0 g	0 g	0 %
Fibre / Fibres	0 g	0 g	
Sugars / Sucres	0 g	0 g	
Protein / Protéines	0 g	0 g	
Vitamin A / Vitamine A			
Vitamin C / Vitamine C			
Calcium / Calcium			
Iron / Fer			
Vitamin D / Vitamine D			
Vitamin E / Vitamine E			15 %

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PRODUCT SPECIFICATIONS / CARACTÉRISTIQUES DE PRODUIT :

Size	16 L Pail	16 L JIB	4/3L JUG	1020 kg Tote	930 kg tote
Richardson Product Number	523 1 47 30	523 1 64 30	521 1 44 10	523 1 69 10	523 1 69 01
UPC	59842 44730	59842 46430	59842-10167 3	-	-
SCC	00059842447306	00059842464303	1 00 59842 14410 3	-	099482403119
Unit Size	16L	16L	3L	1020 kg	930 kg
Unit Dimensions LxWxH *(cm)*	28.0 x 37.5	24.8 x 38.4	16.41 x 27.15	113.5 x 121.92	111 x 103
(inches)	11.0 x 14.8	9.8 x 15.1	6.46 x 10.69	*44.7 x 48.0*	43.7 x 40.6
Units / Case	N/A	N/A	4	N/A	N/A
Case Weight	15.50 kg	15.60 Kg	11.63 Kg	1020 kg	984.90 Kg
Case Cube	23090.7 cm3	23617.5 cm3	1521.46 cm3	1707599 cm3	1280496 cm3
Case Dimensions LxWxH *(cm)*	28.0 x 37.5	24.8 x 24.8 x 38.4	27.10 x 33.70 x 27.30	123.4 x 113.5 x 121.92	112 x 111 x 103
(inches)	11.0 x 14.8	9.8 x 9.8 x 15.1	10.67 x 13.27 x 10.75	*48.6 x 44.7 x 48.0*	44.1 x 43.7 x 40.6
Units / Pallet	48	60	60	1 X 1	1 X 1
Pallet Pattern	16 x 3	20 x 3	5 x 12	1 X 1	1 X 1
Pallet Dimensions LxWxH *(cm)*					
(inches)					
Pallet Cube					
Pallet Weight					

Appendix III: Data sheet of regular canola oil

RICHARDSON

TECHNICAL DATA SHEET

CANOLA HARVEST 100% CANOLA OIL

PRODUCT SPECIFICATIONS / CARACTÉRISTIQUES DE PRODUIT :					
Size	4/4L JUG	6 /1.42L Bottle	6/946mL Bottle	8 /473 mL Bottle	
Richardson Product Number	523 1 45 30	521 1 49 10	521 1 09 10	521 1 07 10	
UPC	59842-44530	0 59842 14810 4	0 59842 10810 8	0 59842 10510 7	
SCC	099482403119	1 00 59842 14910 8	1 00 59842 10910 2	1 00 59842 10710 8	
Unit Size	4L	1.42 L	946 mL	473 mL	
Unit Dimensions LxWxH (cm)	16.59 x 32.00	10.74 x 29.20	10.74 x 20.70	7.53 x 20.91	
(inches)	6.53 x 12.60	4.23 x 11.50	4.23 x 8.15	2.96 x 8.23	
Units / Case	4	6	6	8	
Case Weight	15.49 kg	8.20 kg	5.50 kg	3.67 Kg	
Case Cube	1831.10 cm3	964.36 cm3	680.34 cm3	462.81 cm3	
Case Dimensions LxWxH (cm)	27.40 x 33.80 x 32.40	24.60 x 22.00 x 29.20	24.60 x 22.00 x 20.60	23.60 x 15.60 x 20.60	
(inches)	10.79 x 13.31 x 12.76	9.69 x 8.66 x 11.50	9.69 x 8.66 x 8.11	9.29 x 6.14 x 8.11	
Units / Pallet	48	100	100	180	
Pallet Pattern	4 x 12	5 x 20	5 x 20	6 x30	
Pallet Dimensions LxWxH (cm)					
(inches)					
Pallet Cube					
Pallet Weight					

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Appendix III

Preparation instructions of the low-MUFA high-SFA oil blend (control) treatment

COMIT II: Composition of control oil

Amount for 60g shake (3000kcal)	
Oil	**Grams (g)**
Safflower oil obtained from eSutras	20.94
Butter oil/Ghee, Verka brand obtained from Real Canadian Superstore	21.6
Coconut oil obtained from eSutras	9.6
Flaxseed oil from Shape Foods	7.86
Total	60

1. Add specific 0.87696 kilograms (kg) of safflower oil into 20L pail
2. Add specified 0.42185 kg of flaxseed oil into same pail
3. Add specified 1.48176 kg of Ghee into same pail
4. Add specified 0.24343 kg of coconut oil into same pail
5. Blend using industrial immersion blender in pail
6. Cap and label as control treatment and date of mixing

Appendix III

Fatty acid composition of the low-MUFA high-SFA oil blend (control) treatment

Fatty acid	Amount in shake %
Saturated fatty acids	40.2
Long-chain fatty acids	22.1
Medium-chain fatty acids (C10:0-C15:0)	18.1
Monounsaturated fatty acids	22.0
Polyunsaturated fatty acids less alpha-linolenic acid	29.6
Alpha-linolenic acid	8.2

Appendix III

Diet sheet/sample menu provided for participants as the background diet

Based on 3000 kcal: 35% fat, 50% carbohydrate, and 15% protein of total energy

PARTICIPANT	
BREAKFAST	**gm**
English muffin, whole grain	90
Egg substitute	75
Canadian bacon	38
American cheese	28
Shake	330
LUNCH	
Chicken salad:	
Chicken, cooked, diced	75
Scallions, chopped	6
Grapes, halved	45
Light Mayo	16
Non fat sour cream	16
Lemon juice	6
Lettuce, shredded	35
Tomatoes	35
Pita bread, whole wheat	105
Melon balls, frozen	285
Crackers, whole wheat, low fat	42
DINNER	
Jambalaya	220
Chicken breast, cooked	120
Turkey sausage	40
Lettuce, romaine	85
Tomatoes	62
Carrots, grated	55
Reduced fat Italian dressing	30
Dinner roll	55
Margarine	8
Applesauce **(1 container=112g)**	112
SNACK	
Banana muffin **(1 muffin = 43 g)**	86
Shake	330